DULIN, MAX W., M.S. An Investigation of Paedomorphic Secondary Xylem and Secondary Woodiness in *Xanthorhiza simplicissima*, *Coreopsis gigantea*, and *Mahonia bealei*. (2008)
Directed by Dr. Bruce K. Kirchoff. 222 pp.

The stem and secondary xylem anatomy of *Xanthorhiza simplicissima*, *Coreopsis gigantea*, and *Mahonia bealei* are described and characterized in the study. One focus of the study was to determine the degree of paedomorphosis in the secondary xylem of the three plants. Another goal was to assess their woodiness in a phylogenetic context to determine how their woodiness was derived (whether from woody or nonwoody ancestors). Quantitative measurements and observations from slides and macerations were done using light microscopy and confocal microscopy. The secondary xylem of all three species has a degree of paedomorphosis. *Coreopsis gigantea* has the greatest degree. It possesses vessel elements whose length either decreases or remains the same across the xylem, vessels with pseudoscalariform lateral wall pitting and simple perforation plates, raylessness, libriform fibers, and abundant xylary parenchyma. *Xanthorhiza simplicissima* has an intermediate degree, possessing decreasing vessel element lengths, libriform fibers, and upright ray cells. *Mahonia bealei* has only the paedomorphic characteristics of stable vessel element lengths and libriform fibers. Phylogenetic analysis indicates the species are secondarily woody. A major conclusion is that the degree of paedomorphosis reflects the species release from mechanical requirements.

AN INVESTIGATION OF PAEDOMORPHIC SECONDARY XYLEM AND SECONDARY WOODINESS IN *XANTHORHIZA SIMPLICISSIMA, COREOPSIS GIGANTEA*, AND *MAHONIA BEALEI*

by

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A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2008

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APPROVAL PAGE

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ACKNOWLEDGMENTS

I thank Dr. Bruce K. Kirchoff for his role as advisor, and his assistance throughout the research process. His willingness to share his technical expertise and writing skills has greatly improved the thesis. I also appreciate the help of my committee members, Dr. David Remington and Dr. Dennis LaJeunesse, who provided additional guidance.

There are several persons I would like to acknowledge for their help in obtaining plant specimens. Pete Diamond, Herbarium and Rare Plant Curator at the North Carolina Zoological Park, assisted me with identifying *Xanthorhiza simplicissima* specimens and some of their local growing sites. In addition, I am grateful to Bruce G. Baldwin and Holly Forbes, both with the University of California at Berkeley, for their efforts in providing specimens of *Coreopsis gigantea*. Last, Dr. Matina Kalcounis-Rueppell of the University of North Carolina at Greensboro allowed me to collect samples of *Mahonia bealei* from her family's yard, which I appreciate.

Funding for various supplies and chemicals, as well as confocal microscope use, came through Student Research Support grants provided by the University of North Carolina at Greensboro.

In closing, I thank Dr. Sherwin Carlquist, now retired from the University of California at Santa Barbara, for his extensive botanical research, which forms the foundation for the thesis, as well as his encouragement via e-mail.

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CHAPTER I

INTRODUCTION

Much of the research conducted by plant anatomists on the secondary xylem and other associated tissues such as the vascular cambium has been skewed towards typical woody dicotyledons like trees. This focus is seen in many of the articles of the IAWA (International Association of Wood Anatomists) Journal. Another example of this focus is the website InsideWood, an online wood anatomy information and image database supported by the Department of Wood and Paper Science at North Carolina State University. Part of this orientation is due to the sheer usefulness and the commercial applications of secondary xylem (i.e. wood) found in many typical woody dicotyledons. Thus, much research has been geared towards understanding stem anatomy in order to advance various commercial applications using wood.

Other research by plant anatomists has had a less utilitarian focus. Instead it has been oriented towards answering scientific questions that are interesting, but may have no commercial application. An example of this is the effort of D.A. Kribs to develop a broad classification of rays. But even research like this has been affected by the focus on typical woody dicotyledons. Kribs' ray classification system was based on a study using a collection of mostly typical dicotyledonous woods (Carlquist 2001).

However, despite the emphasis on typical woody dicotyledons, plant anatomists have also been interested in the secondary xylem of plants that are not typical

woody dicotyledons. These are plants that exhibit patterns of woodiness and secondary growth not seen in typical woody dicotyledons. For example, *Arabidopsis*, an herbaceous plant, has been used recently as a genetic model for both primary and secondary xylem development (Chaffey et al. 2002; Nieminen et al. 2004). There has also been increased interest in anomalous secondary growth and cambial variants which differ from most dicotyledons (Rajput and Rao 1999).

This, of course, is not just a recent phenomenon. As far back as the 19th century, both Darwin and Wallace debated about how woody plants could appear on islands. This phenomenon is puzzling because environmental conditions limited the successful dispersal of seeds from trees and other typical woody plants to the islands (Darwin 1859; Wallace 1878). Others such as Chrysler (1937) and Carlquist (1962) studied plants with secondary xylem that had characteristics not usually seen in typical woody dicotyledons. Interest in these areas of research has produced the theory of paedomorphosis in secondary xylem. It has also led to the concepts of insular woodiness and secondary woodiness.

In the secondary xylem, paedomorphosis refers to certain characteristics that are usually associated with the primary xylem in typical woody dicotyledons. Paedomorphic features in the secondary xylem are most often found in less woody plants that have shrubby, herbaceous, or lianoid forms. Insular woodiness occurs when plants from predominantly herbaceous groups have increased woodiness when found on islands or in mountainous equatorial areas. Secondary woodiness means that woodiness is derived from herbaceous ancestors.

Paedomorphosis in secondary xylem and the related concepts of insular woodiness and secondary woodiness were explored in depth in this study. In particular, the secondary xylem of three species (*Xanthorhiza simplicissima*, *Coreopsis gigantea*, and *Mahonia bealei*) was examined for characteristics of paedomorphosis. In addition their phylogenies were reviewed for evidence of secondary woodiness.

Xanthorhiza simplicissima, also known as yellowroot, is a member of the family Ranunculaceae, which falls under the order Ranunculales. It is a perennial dicot found as a small deciduous shrub which can reach 90 cm tall. Its leaves are alternate, pinnately or bipinnately compound, and usually divided into three to five leaflets which are serrate to deeply toothed, with long slender petioles (see Figures 1. and 2.).

The bark is gray-brown and smooth, with the inner bark yellow-colored. The flowers of the plant are yellow to purple-brown, have five petals, and appear in early spring. It usually is located in shady damp woods near water, and ranges from New England to northern Florida as a native plant. It was widely used for medicinal purposes by Amerindians. More recently it was used in folk medicine in the American South (Reed 2004; Seiler et al.2008).

Another of the plants in the study, *Coreopsis gigantea*, is also known as giant coreopsis. It is a member of the family Asteraceae, which is in the order Asterales. A perennial dicot, it grows as a native shrub ranging from 0.45 m to 1.2 m tall on rocky ocean cliffs and dunes of the coast sage shrub and coastal strand plant communities of California. It is drought tolerant, grows best under full sun, and lives in well-drained soil. The leaves are fern-like and pinnately divided into linear segments clustered at the ends

of the branches. It blooms with flowers that resemble the common daisy during March to May. These are formed with large radiate yellow flowering heads in cymes on long scapiform penduncles. The trunk of *Coreopsis gigantea* is sturdy like a small tree. It usually has a cluster of small branches at the top (see Figures 3. and 4.) (Hall 1993; Kartesz 2008).

Mahonia is a genus that includes about seventy species in the family Berberidaceae, which is considered to be a sister family to Ranunculaceae (Hoot et al. 1999). *Mahonia* is an evergreen shrub native to eastern Asia and North and Central America. Several species are grown ornamentally, and cultivated throughout the United States in suburbia.

Perhaps *Mahonia bealei* is a "typical" example of cultivated *Mahonia*. *Mahonia bealei* is usually grown as a garden shrub. It is an evergreen perennial dicot shrub native to eastern Asia, growing to 3.7 meters tall. Its leaves are alternate and pinnately compound, and composed of nine to fifteen oppositely growing ovate leaflets that have spiny dentate margins. The yellow flowers emerge in spring and produce blue-black berries covered in a waxy white bloom, beginning in late summer. It grows in a rounded, open, irregular habit with upright branching (Kling et al. 2008). This is the species used in the thesis as a representative of the genus (see Figures 5. and 6.).

The underlying issue that led to the project itself is that no one has looked at the secondary xylem of *Mahonia bealei* in depth in relation to paedomorphosis, although it is considered to have secondary woodiness (Loconte and Estes 1989; Qui et al. 1993; Carlquist 1995). In addition, while some paedomorphic features of the xylem have been

discussed in the literature for *Xanthorhiza simplicissima* (Carlquist 1995) and for *Coreopsis gigantea* (Carlquist 1985), this information is incomplete. Also, in both cases whether secondary woodiness exists is not resolved completely by the literature. Carlquist (1995) states that *Xanthorhiza simplicissima* has paedomorphic rays, which makes it possible to interpret its wood as being secondarily woody. However, he does not discuss its phylogeny in relation to secondary woodiness. Both Carlquist (1974; 1985) and Thorne (1969) consider *Coreopsis gigantea* as an example of insular woodiness. Neither addresses the issue of secondary woodiness.

Given these issues, one goal of the thesis was to describe and characterize the secondary xylem of the three species, *Xanthorhiza simplicissima*, *Coreopsis gigantea*, and *Mahonia bealei*. Another goal was to determine the extent to which the secondary xylem of the three plants adheres to the descriptions of paedomorphic characteristics in the literature, with an emphasis on *Mahonia bealei*. Finally, the last goal was to place the woodiness of the plants (especially *Mahonia bealei*) into the context of their phylogenetic relationships. By doing this, it could be determined whether they were secondarily woody. These goals were accomplished by using light and confocal microscopy to look at the xylem. In addition a review of the literature enabled researching closely related species for any indication of woodiness to provide a phylogenetic perspective.

It was hypothesized that *Mahonia bealei*, like *Xanthorhiza simplicissima* and *Coreopsis gigantea*, would show the characteristics of paedomorphosis in the secondary xylem. It was also hypothesized that *Mahonia bealei*, as well as the other two plants,

would possess secondary woodiness. This would be demonstrated by the context of their phylogenetic relationships.

CHAPTER II

REVIEW OF THE LITERATURE

The Stem Structure and Wood Anatomy of Ranunculaceae and *Xanthorhiza simplicissima*

Stem Anatomy of the Ranunculaceae

Xanthorhiza simplicissima belongs to a genus containing only one species (sometimes referred to as monospecific). It is a member of the family Ranunculaceae, which contains 47 genera and 2000 species (Judd et al. 2002). The family includes herbs, shrubs, aquatic plants, and some vines, although most species are herbaceous. Members of the family are widely distributed, occurring mostly in the Northern Hemisphere in temperate and boreal regions. However, they also extend into the tropics (Metcalf and Chalk 1950; Judd et al. 2002).

In the Ranunculaceae, the stems usually are circular in cross-section. Cork is rarely formed. However, there is deep seated cork in some species. Interxylary cork is found in one species of both *Aconitum* and *Delphinium* (Metcalfe and Chalk 1950, 1988, 1989).

In transverse sections of the stem in the Ranunculaceae, vascular bundles often occur collaterally and are widely spaced (Metcalfe and Chalk 1950). They may also occur in several concentric rings in some genera. Sometimes they are randomly scattered (Judd et al. 2002). The bundles typically have xylem strands which are concave on the side

facing the phloem. As a result the phloem is partly surrounded by the xylem. Arcs of fibers may also be present just outside the phloem. In the genus *Paeonia* the xylem strands are flattened along the outer edge facing the phloem. Fibers connect the xylem of the adjacent vascular bundles. In some genera vascular strands may be completely surrounded by sclerenchyma sheaths, as seen in some species of *Ranunculus*. In woody genera such as *Clematis* and *Naravelia* an interfasicular cambium is frequently absent. In these genera the vascular bundles are separated by the primary rays. Secondary rays (i.e. rays that are produced by secondary growth) appear within the bundles. In some species of *Clematis*, secondary vascular bundles develop from an interfasicular cambium (Metcalfe and Chalk 1950, 1989).

Pith tissue found in members of the Ranunculaceae generally is composed of parenchyma, and frequently has many intercellular spaces. In many herbaceous species the pith is hollow (Metcalfe and Chalk 1950).

Medullary bundles (i.e. vascular bundles located in the pith) are found in some genera, such as *Anemone*, *Anemonopsis*, *Delphinium*, and *Glaucidium*. When they are found, they are arranged in some variation of the medullary bundle pattern seen in *Glaucidium*. In *Glaucidium* a circle of medullary bundles reaches from the stem base to the floral receptacle (the part of the pedicel where the flower parts attach). One variation of the pattern is that leaf trace strands enter the pith. Strands from the perianth (the sepals and petals of the flower) remain outside the pith. In other genera, the perianth strands enter the pith.

Another variation in medullary bundle patterns is found in *Cimicifuga japonica*. In this species both the cauline vascular bundles (bundles formed entirely from bundles that remain in the stem and never enter leaves) and the vascular bundles from lateral branches are medullary. Primary medullary rays are typically wide (Metcalfe and Chalk 1950, 1989).

The herbaceous members of Ranunculaceae usually have vessels with simple perforation plates. However, *Paeonia*, is an exception to this rule. It has vessels with scalariform perforation plates. In general, vessel scalariform perforation plates in Ranunculaceae are uncommon. Helical thickening on the surfaces of the vessels is found in many species. Vessels are typically distributed in a ring porous pattern. Mean vessel element length in the Ranunculaceae is 195 μm (Carlquist 1995 b.).

Fibers found in the wood have distinctly bordered pits. They can be storied, as well as parenchyma located in the wood (Metcalfe and Chalk 1950, 1989).

In some species such as *Clematis*, the xylem may be furrowed. In the instances where furrowed xylem exists, the furrows are filled with either rays or groups of phloem cells. If the furrows are shallow, the xylem can be described as lobed. In many herbaceous species the xylem of the vascular bundles may be described as v-shaped (Metcalfe and Chalk 1950, 1989).

Although these xylem characteristics are generally true for the family, there is much variation in wood structure among genera, as illustrated in *Clematis* and *Paeonia*. In *Clematis* the vessels have simple perforation plates. The vessel elements are moderately short in length (mean of 250-500 μm). They tend to be large, although in late

wood they are smaller. They have helical thickenings and have alternate pitting between vessels. In addition, they are distributed in a ring porous pattern, and are grouped either tangentially or irregularly. The parenchyma is storied and paratracheal. The rays are all multiseriate, heterogeneous, and very high (greater than 2 cm) and wide (up to twelve cells wide). Rays have a density of 1-2 per mm. The fibers in *Clematis* have numerous simple pits (libriform fibers) and are storied. They typically are extremely short (mean length of 370-500 μm). The fiber walls are moderately thin (Metcalfe and Chalk 1950).

In contrast, the wood of *Paeonia* has small vessels with scalariform perforation plates, usually with two to five thick bars. The vessel elements are of medium length (mean length of 400 μm). They have opposite pitting between other vessels and between vessels and the ray cells. They are mostly solitary, and are distributed in a ring porous or semi-ring-porous pattern. Parenchyma is very sparse, and only found rarely among the fiber cells. Rays in *Paeonia* are frequently uniseriate. They are also homogenous, and are composed of square to upright cells. In addition, they are not very high (less than 1 mm), and are up to three cells wide. They have a density of about 12 per mm. Fibers in *Paeonia* are numerous. They have bordered pits, and are short (mean length of about 550 μm). The fiber walls are thick (Metcalfe and Chalk 1950).

Both solitary crystals and cluster crystals made of calcium oxalate are found in some genera (Metcalfe and Chalk 1950, 1989). Also, triterpenoid saponins, various alkaloids, and a lactone glycoside called ranunculin are found in some members of the family (Judd et al. 2002). Berberin is found in *Coptis trifolia* (Metcalfe and Chalk 1950,

1989; Millspaugh 1974) and *Xanthorhiza simplicissima* (Millspaugh 1974; Okunade et al.1994).

Stem Anatomy of Xanthorhiza simplicissima

Many members of Ranunculaceae are herbs. Some are herbaceous perennials which have aerial stems that emerge from perennial underground tubers or rhizomes. Many of the members with aerial growth forms have annual stems that are relatively short-lived, and have only primary growth. Any secondary growth is confined to underground organs in the majority of the members. However, the genera *Clematis* and *Xanthorhiza* are exceptions to this, for they have above ground woody stems. However, many of the woody stem structures are not self-supporting. These forms include lianas, creeping plants, and small shrubs which are either partially self-supporting or procumbent (Rowe et al. 2004; Isnard et al. 2003).

Xanthorhiza simplicissima is a small shrub which forms an extensive network of rhizomes that produces weakly upright woody stems. The stems are sparsely branched. They are rarely more than a meter in height, and are limited to a diameter of 3-10 mm. Younger stages of the stems are relatively erect, while older stems may become partly procumbent. Very young stems have a ring of lignified primary fiber bundles similar to the organization in species of *Clematis*. Older stems have dense wood with small groups of relatively narrow diameter vessels, and conspicuous large unlignified rays. The dense wood found in *Xanthorhiza simplicissima* is rare in the Ranunculaceae (Rowe et al. 2004; Isnard et al. 2003).

Xanthorhiza has relatively uniform vessel diameters, and lacks very wide vessel elements. The mean vessel diameter is 20 μm. The vessel walls are uniformly thin, with a vessel wall thickness of 1 μm. Mean vessel length is 167 μm. Only simple perforation plates occur in the vessels, and the lateral wall pitting of vessels consists of alternate circular to oval pitting. There is no helical sculpturing on the vessel walls. The vessels are restricted to the central portions of fascicular xylem. Highly pronounced growth rings are present, with many narrow latewood vessels present. Vessels may contain a yellowish material (Carlquist 1995 c.).

There is a relatively low vessel density in *Xanthorhiza simplicissima*. The mean number of vessels per group is 4.5 vessels. In transections where rays are included the mean number of vessels per mm^2 is 198 vessels. It averages 285 vessels per mm^2 in sections of fascicular areas only. The mesomorphy ratio, where vessel diameter times vessel element length is divided by number of vessels per mm^2, and then multiplied by vessel element length, is 11.7 (Carlquist 1995 c.).

The mesomorphy ratio is intended to provide an indication of actual hydraulic conductivity, rather than theoretical hydraulic conductivity (Carlquist 1985). Theoretical hydraulic conductivity is often computed using the Hagen-Poiseuille equation, which was developed independently by Hagen and Poiseuille from experimentation during the 1830's (Tyree and Zimmerman 2002). The equation itself is based on water flow through smooth walled capillaries with circular cross-sections (Tyree and Zimmerman). Another measure of theoretical hydraulic conductivity that is used sometimes is flow rate, which is proportional to the fourth power of the radius of the capillary (Carlquist 1985; Tyree

and Zimmerman 2002). Carlquist used the mesomorphy ratio because it provides a more conservative value of conductivity than flow rate or the Hagen-Poiseuille equation (Carlquist 1985). Several studies have shown that predictors of hydraulic conductance such as the Hagen-Poiseuille equation consistently overstate conductivity when tested against actual conductivity (Carlquist 1985). Higher values (such as that of *Coreopsis gigantea*) are predictors of succulence. For example, members of Cactaceae included in the study had mesomorphy ratios that ranged from 94.6 to 514 (Carlquist 1985).

Rays in *Xanthorhiza* are composed of square to upright cells (as seen in tangential sections), which may contain starch grains. In the literature square cells are considered the morphological equivalent of upright cells (Carlquist 1995 c.). In dicotyledons ray cells in the pith [pith rays are also known as medullary rays or the interfascicular region, which is the tissue found between the vascular bundles, or the Latinate fascicles (Esau 1977)] tend to be more upright than the ray cells in the secondary xylem. However, sometimes the ray cells in the secondary xylem retain the juvenile characteristics displayed by the pith ray cells, such as ray cell uprightness. In these instances they are called paedomorphic (Carlquist 1962). According to this definition the rays in *Xanthorhiza simplicissima* are Paedomorphic Type II. Paedomorphic Type II is a category in Carlquist's modification to Kribs' (1935) ray classification system (Carlquist 2001). The secondary rays in *Xanthorhiza* are basically extensions of the primary rays (Carlquist 1995 c.).

Kribs' ray classification system is based on a study of a wood collection that contained mostly trees. As a result it does not include categories for ray types found in

less woody growth forms such as woody herbs or rosette trees. Carlquist modified the system to include paedomorphic ray types found in those less woody plants (Carlquist 2001). One of his additions, Paedomorphic Type II rays, is a category for multiseriate rays that consist of upright ray cells, or at least predominantly upright ray cells (Carlquist 2001).

Libriform fibers are the only imperforate tracheary element type in *Xanthorhiza*. These have only simple pits, and are relatively thin walled (3 μm mean wall thickness). The mean length of the libriform fibers is 342 μm, which is relatively short. The ratio of imperforate tracheary element length divided by vessel element length is 2.05. This ratio is typical for species with libriform fibers. If tracheids or fiber-tracheids were present in the wood, a lower ratio (closer to 1.0) would be expected. Libriform fibers are found next to rays, and may contain starch grains (Carlquist 1995 c.).

Axial parenchyma is rare in *Xanthorhiza*. In some members of Ranunculaceae like *Clematis* it may be found in strands of two to four cells. In contrast, in *Xanthorhiza* it is found only in strands of single undivided cells. No storying in axial xylem cells is seen (Carlquist 1995 c).

Secondary phloem is found in small increments. It contains narrower sieve tube elements than found in other Ranunculaceae genera such as *Clematis iringaensis* (Carlquist 1995 c).

The Stem Structure and Wood Anatomy of Asteraceae (Compositae) and *Coreopsis gigantea*

Stem Anatomy of the Asteraceae

Coreopsis gigantea is a member of the family Asteraceae (Compositae), a large family whose species are found in a wide range of habitats and geographic areas. In order to adapt to the different habitats, the family members have evolved into a variety of structural forms: herbs, shrubs, or trees. However most are herbaceous (Heywood and Harborne 1977; Cronquist 1955; Metcalfe and Chalk 1950). Of these forms, the herbs and shrubs tend to be found in regions where there is the greatest phyletic diversity in other taxa. This is frequently the xeric montane areas of subtropical and tropical North and South America. In contrast, trees or tree-like forms tend to be found in areas of low phyletic diversity, such as middle and low tropical or subtropical rain forests. They also occur as insular endemics (Heywood and Harborne 1977).

The anatomical structure of members of Asteraceae reflects this range of habits. The axis of the stem is bounded on the outside by an epidermis formed of dead cells. In some genera, a persistent epidermis, where the epidermal cells grow and divide for several years, is found. The cork, if it is present, is usually superficial. It can be deep seated in some species (Metcalfe and Chalk 1988). The cortex can vary in form. Its outer part may be made up of collenchyma, or chlorenchyma (unlignified tissue containing chloroplasts), or alternating segments of both tissue types. In some species, for example *Aster tripolium*, the cortex may be composed of aqueous tissue (tissue of enlarged cells that contain water). There are also other possible variations. In *Cassinia* the outer cortex

is lignified and is differentiated from the inner cortex (the cells of the inner cortex have thin cell walls) In some species like *Baccharis* sclerosed cells (sclerotic cells, or cells that have hard, lignified walls) are embedded in the cortical parenchyma. Interxylary cork layers have been found in one species, *Artemisia tridentata* (Metcalfe and Chalk 1950; Usher 1966).

In the Asteraceae phloem typically has sclerenchymatous elements like fibers. However in some instances phloem containing lignified companion cells and lignified sieve tubes is also found. Intraxylary phloem is found in the stems of many genera (Metcalfe and Chalk 1989). In species which are herbaceous, the stems usually have a ring of collateral vascular bundles. In other genera which are shrubs or arboreal, there may be closely spaced vascular bundles or a woody cylinder. Another variation is irregularly distributed vascular bundles. In arboreal genera such as the *Senecio*, the stem anatomy is more like that of very large herbs rather than those of reduced trees. In these genera young seedlings have a ring of vascular bundles. In mature stems there is a cylinder of xylem surrounding a large pith, which stores water (Metcalfe and Chalk 1950).

In several genera of Asteraceae vascular bundles located in the cortex are composed of large vascular strands accompanied by arcs of smaller bundles. In other genera such as *Atractylis*, *Centaurea*, and *Cirsium*, every cortical vascular bundle is accompanied by an arc of imperfect strands. These imperfect strands consist only of primary phloem (since they are missing xylem, the strands are considered “imperfect”, just as flowers missing either anthers or carpels are called imperfect). In bicollateral

bundles, as these are sometimes called, the imperfect strand is interior to the xylem of the vascular bundle (Mauseth 1998). Other genera have vascular bundles located in the pith (medullary bundles), which take on a variety of forms. Although these are usually located collaterally, they sometimes are centric. In addition they may be incomplete and consist of phloem only (Metcalfe and Chalk 1950).

Pith tissue in the Asteraceae is also varied in form and structure. Often the pith cells have thin cell walls, but they can have thick walls in some species. The pith can be hollow, septate (as in *Senecio*), or sclerified (as with *Baccharis*) (Metcalfe and Chalk 1950). The width of primary medullary rays may be broad or narrow (Metcalfe and Chalk 1988).

The wood (xylem) of the family Asteraceae also exhibits a range of features. Vessel features, especially, vary according to the type of habitat (Carlquist 1992). Typically the vessels are small in diameter (100 μm to less than 50 μm in some species). They are often found in radial multiples of four or more. Frequently they occur in irregular clusters which can be in a tangential as well as a radial pattern. Vessels are commonly found in semi-ring porous distribution patterns. However they can occur in ring porous distribution patterns in some genera (Metcalfe and Chalk 1950, 1989).

The subunits of the vessels, vessel elements, are usually short to very short, with a mean element length of 200-300 μm (Metcalfe and Chalk 1950). Spiral or helical thickening is often present, especially in species growing in temperate regions (Metcalfe and Chalk 1950, 1989; Carlquist 1992). The perforation plates are usually simple, although a few genera have scalariform or reticulate plates (Metcalfe and Chalk 1950,

1989; Carlquist 1992). Pitting between the vessels is alternate. The pit size ranges from small to moderately large. Pitting between the vessels and neighboring parenchyma cells typically follows the same alternate pitting pattern (Metcalfe and Chalk 1950). Tyloses in vessels occur in some members of the family (Metcalfe and Chalk 1989). Vestured pits or vestured perforation plates may occur in the vessels (Metcalfe and Chalk 1989), although this is disputed in other research (Carlquist 1992).

Rays may be storied, although this feature is absent in most of the family, and only occurs in a few species (Metcalfe and Chalk 1950, 1989; Carlquist 1992). Most often the rays are four to ten cells wide. However, they can be up to eighteen cells wide in some genera. Usually there are few uniseriate rays, but some species have exclusively uniseriate rays. If there are multiseriate rays, they are composed of an irregular mix of upright and procumbent cells (heterogeneous or heterocellular rays) (Metcalfe and Chalk 1950). Rayless species are also found, as well as species with paedomorphic rays (Metcalfe and Chalk 1950; Carlquist 1992). Vasicentric tracheids and intercellular canals in rays are occasionally present (Metcalfe and Chalk 1950, 1989).

Fibers found in the xylem of Asteraceae genera typically have small simple pits (libriform fibers) or pits with very small borders. Fibers with distinctly bordered pits are sometimes found (fiber tracheids). The fibers tend to be medium to very short length (mean 500-1400 μm). Occasionally they are septate (rare or uncommon). They may have spiral thickening. Fibers may also be storied (Metcalfe and Chalk 1950, 1989).

Scanty vasicentric axial parenchyma is found in the Asteraceae (Carlquist 1992). Typically parenchyma in the xylem is sparse and paratracheal. Also the cells usually have

no contents. Most often the parenchyma is found in strands of one or two cells. Storied parenchyma is, however, found in some species of *Artemisia*, *Baccharis*, *Bigelowia*, and a few other genera of Asteraceae (Metcalfe and Chalk 1950, 1989).

The family Asteraceae also has other interesting variations in stem anatomy and structure. For example, various kinds of crystals are found in some genera. Solitary crystals, clusters of crystals, raphides, ascicular crystals, or sphaerocrystals may be present (Metcalfe and Chalk 1989). Cambial variants such as successive cambia and anomalous secondary thickening occur in a few species (Carlquist 1992). For example, in *Bidens rubifolia* and in species of *Mikania*, the xylem is split into sections by wide primary and secondary rays. *Mikania* also has a secondary zone of xylem and phloem originating from the pericycle. A few genera in the Asteraceae tribe Inuleae have anomalous secondary thickening where the primary bundles do not have any cambium. In the genus *Phaenocoma* a supplementary cambium develops later. Also, in some genera a cambial zone is formed around the vascular cylinder, and produces xylem and islands of phloem. Other genera have successive concentric rings of vascular bundles (Metcalfe and Chalk 1950).

Secretory tissue also occurs in the stems of the Asteraceae. Elongated secretory sacs (or cavities) in the pith, phloem, or pericycle of the stem are found in some genera. Secretory sacs are internal spaces surrounded by an epithelium of secretory cells (Fahn 1979). Non-mucilaginous cavities are found in other genera. Radial canals are found in the xylem in some of these genera. Canals with various chemical contents can also be

found in the endodermis of the roots, and the cortex and pith. Laticifers are also found in some species (Metcalfe and Chalk 1989).

Stem Anatomy of Coreopsis gigantea

As part of a study of 207 species of southern California plants, Carlquist (1985) compiled data related to the xylem (wood) of *Coreopsis gigantea*. To do this, he used sections and macerations for light microscopy. A minimum of twenty measurements were collected for each feature to get means.

In *Coreopsis gigantea* he found the mean number of vessels per mm^2 is 51.2 vessels. Mean vessel diameter is 36.7 µm. For the mean vessel diameter, measurements were made on the basis of the lumen, rather than the lumen plus the cell wall (the most commonly used technique). Measurements were taken at the widest point of the vessel. The mean vessel element length is 210 µm (measurements used macerated tissue, and included the caudate tips, or tails, of the elements) (Carlquist 1985).

The mean number of vessels per group is 2.24 vessels. To qualify as a group, vessels had to be in actual contact. For this measurement, a solitary vessel was equal to 1.0, a pair of vessels was equal to 2.0, and so forth. This measurement indicates most vessels in *Coreopsis gigantea* tend to be in groups of two to three (Carlquist 1985).

There were no bars present on vessel element scalariform perforation plates. This means only simple perforation plates were present in the specimens of *Coreopsis gigantea* examined by Carlquist. This is a feature common to many plant species in southern California (Carlquist 1985).

Helical sculpturing patterns of the vessel elements were examined in both early wood and late wood to see if they were present. Helical sculpturing was used as a broader term than helical thickening. It included grooves connecting the pit apertures, as well as the raised ridges between the grooves. No helical sculpturing is present in either the early or late wood in *Coreopsis gigantea* (Carlquist 1985).

The xylem was also studied for the presence of growth rings. Xylem has growth rings which are weakly ring porous (semi-ring porous) in the distribution of vessels, in contrast to diffuse porous (Carlquist 1985).

Carlquist (1985) also looked to see whether tracheids were present, using three mutually exclusive categories: true tracheids, vasicentric tracheids, and vascular tracheids. True tracheids were defined as imperforate tracheary elements in which pits are fully bordered. Vascular tracheids are tracheids present only in the last layers of a growth ring, in situations where the wood has either libriform fibers or fiber tracheids. If libriform fibers or fiber tracheids were present in the wood, but tracheids were mixed with the vessels (i.e.tracheids are found in positions other than on the edges of growth rings), vasicentric tracheids were indicated. No tracheids in any of these categories are present in the species (Carlquist 1985).

In addition, Carlquist (1985) calculated a mesomorphy ratio by dividing the vessel diameter by the number of vessels per mm^2, and then multiplying by vessel element length. For *Coreopsis gigantea*, this computation was (36.7/51.2) x 210, for a mesomorphy ratio of 151. Carlquist (1985) also categorized the plants in his study according to habit. *Coreopsis gigantea* was classified as an herb. Although herbs are

normally annuals, biennials, or short-lived perennials, in the case of *C. gigantea*, the category is used to refer to large plants with a degree of succulence normally associated with herbs. Other plants were categorized as shrubs, subshrubs, trees, and vines (Carlquist 1985).

The Stem Structure and Wood Anatomy of Berberidaceae and *Mahonia bealei*

Stem Anatomy of the Berberidaceae

Mahonia bealei is a member of the family Berberidaceae. The family consists of both herbs and shrubs. It is widespread, occurring mostly in the North Temperate regions. Some Berberidaceae are also found in the Andes of South America. In addition to their wide distribution, the family occupies habitats ranging from forest understories to arid regions and deserts. One distinction shared by the Berberidaceae is the possession of such alkaloids as berberin, an isoquinoline alkaloid which colors the wood yellow (Metcalfe and Chalk 1950; Judd et al. 2002).

There are 15 genera and 650 species in the family, with *Berberis* as the major genus (600 species). Some species of *Berberis* have pinnately compound leaves. These are often now treated as a separate genus and segregated as *Mahonia*, or placed as a subgenus of *Berberis* (i.e. *Berberis* subgenus *Mahonia*). Thus, *Mahonia bealei* and *Berberis bealei* refer to the same plant (Metcalfe and Chalk 1950; Judd et al. 2002; Carlquist 1995). For the purposes of the thesis, the term *Mahonia bealei* will be used, however.

In the Berberidaceae, the cork cells of the stem are usually thin walled with wide lumina. The cork may be superficial, or it may be deep seated. In *Podophyllum*, it is subepidermal (Metcalfe and Chalk 1950, 1988).

In woody genera like *Berberis* and *Mahonia* a ring of fibers, which originates in the pericycle [the ground tissue that lies between the phloem and the endodermis of the stele (Esau 1977)], is located at the periphery of the stem. Vascular bundles occur to the interior of the fibers. The bundles are separated by wide primary medullary rays. Herbaceous or semi-herbaceous genera may have widely scattered vascular bundles in their aerial stems. In other genera such as *Nandina domestica* there may be an intermediate type of vascular bundle, where there is a ring of widely spaced bundles which surrounds a large pith. The structure of vascular bundles in the Berberidaceae is similar to those found in monocotyledons (Metcalfe and Chalk 1950, 1988).

Sieve tubes of the phloem in the Berberidaceae have sieve plates on the beveled ends of the tubes. Sometimes the sieve plates are also located on the lateral walls (Metcalfe and Chalk 1950).

Xylem vessels tend to be very small, averaging 25-50 μm in tangential diameter (Metcalfe and Chalk 1950), with the vessel elements moderately to extremely short (mean length 150-270 μm) (Metcalfe and Chalk 1950). In transection, a mm^2 area contains a mean of 25-50 vessels (Metcalfe and Chalk 1950). Usually the vessels are arranged in an ulmiform pattern (i.e. arranged in irregular concentric, wavy bands), as seen in *Berberis*. They can also be arranged in a radial pattern, as found in *Mahonia tenuifolium* and *Nandina domestica*. Occasionally they occur in either a ring porous or

semi-ring porous pattern. Both patterns are found in some species of *Berberis*. They may also be arranged in a dendrite (branching) pattern. If rays are present, the vessels seldom touch the rays (Metcalfe and Chalk 1950, 1988).

Typically vessels have helical thickening and simple perforation plates. However, scalariform perforation plates, while uncommon, may be seen in *Berberis*. In these instances the plates have very few bars. The perforations are slightly oblique. Intervascular pitting is alternate, with moderate sized pits. Pits connecting vessels to ray cells are rarely found. If they occur, the pitting is similar to intervascular pitting. Often imperfect vessel elements which resemble tracheids with helical thickening are present (Metcalfe and Chalk 1950, 1988).

Fibers in the xylem are moderately to extremely short, averaging 300-750 μm in length. They usually have numerous small simple pits on their radial and tangential walls. The fiber cell walls range from moderately thick to thin. Occasionally septate fibers are found in *Berberis* and *Nandina. S*toried fibers are occasionally found in *Berberis* and *Mahonia* (Metcalfe and Chalk 1950, 1988).

Rays in the Berberidaceae usually are wide, with a range of six to twelve cells. However, the ray width can be much greater in certain species, such as *Berberis kavakana* (up to twenty-five cells wide). Rays also tend to be high. Frequently they are greater than 2 mm high, and sometimes greater than 5 mm high (Metcalfe and Chalk 1950). Tall rays often are subdivided into smaller units.Uniseriate rays (rays which are one cell wide in cross-section) are not common. Instead, multiseriate rays predominate.

Rays usually are homogenous or nearly homogenous (homogenous rays contain only procumbent ray cells) (Metcalfe and Chalk 1950).

Other variations in rays include sheath cells, square cells, and the presence of crystals. In *Berberis darwinii* sheath cells are found in the rays (Metcalfe and Chalk 1950). Sheath cells (sometimes referred to as Hüllzellen) are large ray cells (either erect or square) which form a continuous sheath around the center procumbent cells of a multiseriate ray (Chattaway 1933). In *Mahonia tenuifolium* the rays are composed entirely of small square cells (Metcalfe and Chalk 1950). Rays in *Mahonia* may contain numerous calcium oxalate crystals (Metcalfe and Chalk 1950).

A noticeable feature of the woody genera of the Berberidaceae is that the outer edge of the pith consists of cells with thicker cell walls than the cells in the center of the pith (Metcalfe and Chalk 1950). In addition, parenchyma is absent in the wood (Metcalfe and Chalk 1950).

Stem Anatomy of Berberis, Mahonia, and Mahonia bealei

Carlquist (1995 a.) studied the wood anatomy of the Berberidaceae, including twenty-one species of *Berberis* in the study. He considered *Berberis* and *Mahonia* to be subgenera of the genus *Berberis*, giving nine species of subgenus *Berberis* and twelve species of subgenus *Mahonia*. One of these was *Berberis* (*Mahonia) bealei* (Carlquist 1995 a.). However, to avoid confusion in the thesis and to follow convention, *Mahonia* and *Berberis* will be considered separate genera, rather than subgenera.

In *Berberis* and *Mahonia*, vessels are typically larger in earlywood than in latewood. If vessel diameter is used as a criterion, most of the growth ring consists of

latewood. The narrow vessels of the latewood are intermixed with vasicentric tracheids. Although vessels form a ring porous pattern in *Berberis*, in *Mahonia* a semi-ring porous pattern is common. In some species of *Mahonia* living in desert habitats, growth rings start with narrow diameter vessels, and the vessels become wider later in the season. This occurs because the early growing season in the desert begins with an increase in temperature, but no rise in rainfall amounts. Later in the season rainfall increases, which is reflected in the increase in vessel diameter (Carlquist 1995 a.).

Vessel restriction patterns [a term that describes xylem where vessels occur in the central parts of fascicular areas (Carlquist 2001)] in *Mahonia* vary by species. *Mahonia fremontii*, for example, has libriform fibers adjacent to rays, with vessels in only the central part of fascicular areas. The libriform fibers are not as common in the center of the fascicular areas. In contrast, in *Mahonia nervosa*, the vessels are restricted in wood that is formed earliest in the growth ring. However, this is not seen in larger stems. *Mahonia trifoliata* has vessel restriction in some growth rings, but not in other growth rings in the same specimen. Three members of *Mahonia* (*Mahonia aquifolium*, *Mahonia moranensis*, and *Mahonia nevinii*) have vessel restriction patterns in which there is at least one layer of fiber cells between the rays and the vessels. In both *Mahonia bealei* and *Mahonia haematocarpa*, there are no vessel restriction patterns. In these species vessels are not restricted to the center of the fascicular area (Carlquist 1995).

Vessels are grouped in various patterns in transection. In *Mahonia*, there are either diagonal groups of vessels, as seen in *Mahonia bealei*, *Mahonia haematocarpa*, and smaller stems of *Mahonia nervosa*, or no diagonal grouping of vessels. No diagonal

grouping is seen in the larger stems of *Mahonia nervosa* and *Mahonia moranensis*. In general, the number of vessels within a group is high in both *Berberis and Mahonia*. In *Mahonia bealei*, there is a mean of 11.8 vessels per group, and a mean of 15.5 vessels in *Mahonia* as a whole (Carlquist 1995 a.).

Vessel density was measured by mean number of vessels per mm^2 in transection (Carlquist 1995). For *Mahonia*, the mean is 552 vessels per $mm^{2.}$ *Mahonia bealei* has a mean of 488 vessels per mm^2 (Carlquist 1995). The vessel density found in the Berberidaceae is similar to that found in California dessert shrubs (a mean of 361 vessels per mm^2 area) or chaparral shrubs (a mean of 299 vessels per mm^2 area) (Carlquist 1995).

Vessel element length in the Berberidaceae is relatively short. The mean length for *Mahonia* is 221.0 μm. In *Mahonia bealei* the mean vessel element length is 272.0 μm (Carlquist 1995 a.). Mean vessel diameter is also relatively low in Berberidaceae compared to dicotyledons in general. The mean lumen diameter for vessel elements in the genus *Mahonia* is 9.0 μm. In *Mahonia bealei* the mean lumen diameter is 13.0 μm. Narrow vessel diameter characterizes Berberidaceae from both dry and moist habitats (Carlquist 1995 a.).

The diameter of pit cavities in vessels in Berberidaceae is relatively small. In *Mahonia bealei* the mean pit cavity diameter is 4.0 μm (Carlquist 1995 a.). With a couple of exceptions (*Epimedium pinnatum*, *Leontice leontopetalum*) the Berberidaceae have alternate pitting in the vessels, and the pits are circular to oval. Only simple perforation plates are found in the Berberidaceae, with the exception of the vessels in *Epimedium*

pinnatum. These have scalariform perforation plates as well as simple perforation plates (Carlquist 1995 a.).

Helical sculpturing is present in the vessels, except in *Jeffersonia diphylla* (Carlquist 1995 a.). In *Mahonia*, *Mahonia aquifolium*, *Mahonia bealei*, *Mahonia haematocarpa*, *Mahonia pinnata*, and *Mahonia piperiana* all have helically thickened vessels. They also have grooves connecting the pit apertures in the wider vessels. In *Mahonia bealei* this holds true for the narrow vessels as well (Carlquist 1995 a.).

At least some vessel elements are storied in all species of *Berberis and Mahonia*, as well as *Nandina*. Storying occurs only in wood with relatively short fusiform cambial initials, which form short vessel elements. Since Berberidaceae has relatively short vessel elements, storied vessel elements are common (Carlquist 1995 a.).

Storying of vasicentric tracheids, which are immediately adjacent to the vessels, is also found in *Berberis, Mahonia,* and *Nandina*. Vasicentric tracheids are mixed with vessels in most species of *Berberis* and *Mahonia*. This type of tracheid is abundant in species of *Berberis* and *Mahonia* that possess lots of narrow vessels. In addition, helical thickening in vasicentric tracheids is found in species that have vessels with helical thickening (Carlquist 1995 a.).

Fibers in the genus *Berberis* all have simple pits (or perhaps slightly bordered pits) with oval pit apertures. This feature is common to libriform fibers. They are typically embedded in vessel groups or groups of vasicentric tracheids. Libriform fibers embedded in vessel or vasicentric tracheid groups are usually about the same length as the vessel elements and vasicentric tracheids. In contrast, libriform fibers located

elsewhere in the wood are usually about twice as long as the vessel elements. These libriform fibers may have narrow slitlike pits. In *Mahonia* the mean libriform fiber length is 400 µm. The mean libriform fiber length in *Mahonia bealei* is 541 µm (Carlquist 1995 a.).

Other variations in libriform fibers exist, including the presence of starch, septate fibers, and storying. Starch is found in libriform fibers of all the *Berberis* and *Mahonia* species. Septate fibers are seen only in the genus *Berberis* (Carlquist 1995 a.). Storying in some libriform fibers is found in three species *Mahonia* (*Mahonia fremontii*, *Mahonia haematocarpa*, and *Mahonia nervosa*), as well as a few species of *Berberis* (Carlquist 1995 a.).

True axial parenchyma, which has strands of parenchyma cells in which each cell is surrounded by a lignified secondary cell wall, is found in only one species of *Berberis*, *Berberis paniculata*, and in *Jeffersonia diphylla*. In *Berberis paniculata*, the axial parenchyma takes the form of strands of two to four cells (some of which are septate) in radial sections. In *Jeffersonia* the axial parenchyma is composed of one or two celled strands (Carlquist 1995 a.).

Rays are usually multiseriate in *Berberis*, *Mahonia*, and *Nandina*, with uniseriate rays being very uncommon except in *Jeffersonia diphylla*. In *Berberis* and *Mahonia* the rays begin as multiseriate rays instead of uniseriate or biseriate rays. The rays increase in width as stem diameter increases. In a few species some of the larger rays may become broken up into smaller segments. The mean height of multiseriate rays in *Mahonia* is 2110.0 µm. In *Mahonia bealei*, the mean height of multiseriate rays is 2400.0 µm. The

mean width of rays in *Mahonia bealei* measured at the widest point is 4.4 cells. In comparison the mean width of rays in the genus *Mahonia* is 6.7 cells. No storying is present in rays of any of the Berberidaceae of the study (Carlquist 1995 a.).

Upright ray cells are relatively abundant in two species in *Mahonia*, *Mahonia nervosa* and *Mahonia pinnata*. This is true also for several species in *Berberis*. In most species of Berberidaceae, the ray cell wall thickness range 1.8-2.2 µm. However, there are thinner ray cell walls in a few Berberidaceae species, including *Mahonia moranensis* and *Mahonia piperiana*. Except for *Jeffersonia diphylla*, where only the primary cell walls are lignified, all the Berberidaceae species have lignified secondary cell walls in the ray cells. In addition, tangentially oriented ray cell walls in Berberidaceae have more pitting than radially oriented ray cell walls (Carlquist 1995 a.).

Starch and calcium oxalate crystals are present in the Berberidaceae. Starch is found in both libriform fibers and ray cells in all the Berberidaceae species. In *Mahonia* as well as *Nandina* the storage of starch may be related to the rapid periods of growth. Calcium oxalate crystals, which take the form of large rhomboidal crystals, occur in the ray cells of six species of *Mahonia*, including *Mahonia bealei.* This is also true for one species *Berberis*, *Berberis corymbosa*, as well as *Nandina* (Carlquist 1995 a.).

Just like his other studies, one including *Coreopsis gigantea* (Carlquist 1985) and another including *Xanthorhiza simplicissima* (Carlquist 1995 c.), Carlquist (1995 a.) calculated mesomorphy ratios, a measure of hydraulic conductivity, in his study of the Berberidaceae. As mentioned previously, the mesomorphy ratio is the mean vessel diameter times mean vessel element length, divided by the mean number of vessels per

mm^2. He calculated a mesomorphy ratio of 7.2 for *Mahonia bealei*, and a mean mesomorphy ratio of 5.8 for *Mahonia*. The low mesomorphy ratios found for *Mahonia*, together with the presence of large groupings of vessels in latewood where the vessels are accompanied by libriform fibers rather than tracheids, indicate a greater degree of xeromorphy for *Mahonia*. That is to say, *Mahonia* has morphological characters which help to reduce water loss (Carlquist 1995 a.).

Paedomorphosis, Insular Woodiness, and Secondary Woodiness

The theory of paedomorphosis in secondary xylem and the terms insular woodiness and secondary woodiness are related concepts which are complex, nuanced, and sometimes controversial. In addition (and unfortunately), they are not always well defined and used consistently in the literature. Sometimes they are conflated with each other, even though they are not the same, and have important differences. For instance, a plant with certain anatomical characteristics in the secondary xylem may be determined to have paedomorphic wood. Based on this, some researchers imply (or come perilously close) that it must have secondary woodiness (Carlquist 1995 c.; Lens et al. 2005 a., b.) However, without morphological or molecular knowledge about its ancestors, it cannot be said definitively to be secondarily woody. That decision, whether it is secondarily woody, must be made in the context of its phylogenetic relationships (Carlquist 1962). There is similar confusion about insular woodiness. Since insular woodiness can originate from either herbaceous ancestors or extinct woody ancestors, decisions about its origin must be made in a phylogenetic context (Kim et al. 1996). Due to the inherent complexities of these concepts, they must be clearly understood.

Paedomorphosis

In evolutionary theory, paedomorphosis is one of the possible end results of a phenomenon called heterochrony. Heterochrony is broadly defined as the change in both the rate of development of ancestral characters, and the timing of the appearance of the characters during an organism's development (de Beer 1930; Gould 1977; McNamera 1986). At the level of the individual organism, variation of growth rates as well as the length and timing of the period when growth occurs affect the relationship between size and shape of particular structures (McNamera 1986). At the level of the collective organisms (i.e. the species level), the variations in the rate of development and the timing may provide some adaptive advantage, which may be selected for, thus shaping evolution (McNamera 1986; McNamera and McKinney 2005).

Heterochrony is split into two alternative end results, paedomorphosis and peramorphosis. Paedomorphosis results if an organism passes through fewer developmental stages than its ancestor, resulting in the adult form of the organism possessing morphological characteristics which occurred in the juveniles of its ancestor (McNamera 1986). Peramorphosis, on the other hand, results if the organism passes through more developmental stages than its ancestor, leading to the adult form of the organism developing beyond the ancestor (McNamera 1986). Both paedomorphosis and peramorphosis can be achieved by more than one way. For example, paedomorphosis can happen by the reduction of the rate of morphological development through the juvenile growth stage (neotony), by early sexual maturation (progenesis), or by the delay of the beginning of morphological development (post-displacement) (McNamera 1986).

Paedomorphosis has been applied beyond general evolutionary theory to the study of specific animals and plants. In botany it has been used to explain the anatomy of the secondary xylem found in certain dicotyledons. Although perhaps Carlquist (1962) is best known for developing the paedomorphosis theory in plant anatomy, the concept of plant anatomy related paedomorphosis was discussed in the literature much earlier than his seminal work. Chrysler's study (1937) of *Zamia* (a genus of Cycadaceae) provides an early glimpse of the ideas that Carlquist would refine.

When Chrysler investigated xylem taken from a mature specimen of *Zamia floridana*, he found tracheids with scalariform lateral wall pitting throughout the xylem. This is in contrast with the other genera in the order Cycadales, which have either tracheids with smaller bordered lateral wall pits, or tracheids which make a transition from scalariform lateral wall pitting (tracheids near the pith) to tracheids with smaller bordered pits (tracheids further from the pith). Thus, in the last pattern of tracheid lateral wall pitting, as the cycad grows, the pitting patterns change from scalariform pitting in the earliest formed xylem to small pits in the later, more mature xylem (Chrysler 1937).

Since *Zamia floridana* has a tuberous stem or caudex, Chrysler decided to look at additional tracheid pitting by using xylem from two other species of *Zamia* which possess well defined trunks, *Zamia pseudoparasitica* and *Zamia tuerckheimii*. The xylem from the mature specimen collected in Costa Rica, *Zamia pseudoparasitica*, showed a pattern of tracheids with scalariform lateral wall pitting close to the pith. In addition it had tracheids with transitional lateral wall pitting further out in the stem. Tracheids found closer to the phloem had circular bordered pits. The trunk of a young specimen from

Guatemala, *Zamia tuerkeimii*, revealed only tracheids with scalariform lateral wall pitting (Chrysler 1937).

These findings indicate that tracheids with scalariform lateral wall pitting are normally found only in immature cycads or in the earliest xylem growth of more mature cycads. Based on this, Chrysler concluded that the tuberous species, *Zamia floridana*, is a persistent juvenile with respect to its xylem. It has xylem which is immature: the occurrence of only tracheids with scalariform wall pitting indicates an arrested development in the xylem. Although he did not term it paedomorphosis, his observations are essentially in agreement with the theory formulated later by Carlquist (Chrysler 1937).

Carlquist (1962) developed a theory of paedomorphosis applied to the secondary xylem of dicotyledonous plants. The theory is an effort to explain why the anatomy of the secondary xylem of some less woody plants with shrubby, herbaceous, or lianoid forms does not fit the broad anatomical trends usually seen in the secondary xylem of typical woody dicotyledons. His hypothesis is that the anatomy of the xylem of many less woody plants does not fit the general anatomical trends because of paedomorphosis. In the case of secondary xylem, paedomorphosis means that certain features of the primary xylem of the plant stem are protracted into the secondary xylem during development. Thus the secondary xylem of plants with paedomorphic wood shows anatomical characteristics usually associated with the primary xylem in typical woody dicotyledons. Paedomorphic secondary xylem may appear to be permanently "juvenile" compared to that of typical woody dicotyledons, or else may lose those "juvenile" characteristics slowly as

development of the secondary xylem continues over time. In less woody plants paedomorphic secondary xylem retains "juvenile" characteristics (i.e. those of the primary xylem) because the plants are either moving towards, or away from, true woodiness (Carlquist 1962).

It is generally acknowledged that certain anatomical trends among xylary cell types occurred during evolution in typical woody dicotyledons, especially among vessels, fibers, and rays. When vessels evolved from tracheids (which are generally much longer, narrower, and angular in cross-section than vessel elements), they became more specialized over time, and as specialization proceeded, became shorter, wider, and less angular (Frost 1930 a.; Esau 1977). They also evolved from possessing scalariform perforation plates with many bars, to scalariform perforation plates with only a few bars, to simple perforation plates in the most specialized vessel elements (Frost 1931, 1930 b.; Esau 1977). Lateral wall pitting also changed during the evolution of vessel elements. Scalariform lateral wall pitting characterized vessel elements found in early dicotyledons. Over time, however, scalariform pitting evolved into opposite pit pairs, and then into alternate pitting between adjacent vessels (Frost 1931; Esau 1977)..

Fibers were another cell type that evolved from tracheids. In general, fibers evolved thicker cell walls and longer cell bodies than tracheids. Fiber-tracheids are presumed to have evolved first from tracheids, and evolved smaller bordered pit cavities and thicker cell walls. Libriform fibers, the most specialized derivation, developed the thickest cell walls and longest cell body of the three cell types, and also evolved narrow borderless pits (i.e. simple pits) (Esau 1977; Mauseth 1988).

Rays in dicotyledons evolved from heterogeneous rays, which contain both upright ray parenchyma cells and procumbent (wider than they are tall) ray parenchyma cells, into the more specialized homogenous (homocellular) rays. Homogenous rays contain only procumbent ray cells (Kribs 1935; Esau 1977). These general trends can be seen in Kribs' (1935) ray classification system. For example, in Kribs'system, Heterogeneous Type I rays are considered the most primitive (i.e. least specialized), and consist of both uniseriate and multiseriate rays. In Heterogeneous Type I rays, there are tall, numerous uniseriate rays made up of large upright ray cells, which are completely separate from the multiseriate rays. The multiseriate rays in Heterogeneous Type I rays consist of a multiseriate portion, made of both upright and procumbent ray cells, as well as uniseriate wings made up of upright ray cells, which are attached to the multiseriate rays (Kribs 1935; Carlquist 2001). Homogenous Type III rays in Kribs' system are the most specialized, and have only uniseriate rays of procumbent ray parenchyma cells (Kribs 1935; Carlquist 2001).

The least specialized (or more primitive) of the xylary cell characteristics tend to be found in the primary xylem of typical dicotyledonous woody plants. Thus, generally longer vessel elements with scalariform lateral wall pits (with wide apertures), thin walled fibers with wide diameters (or parenchyma only, in addition to vessels and tracheids), and tall rays with cells that tend to more erect, may be found in primary xylem (Carlquist 2001).

The reason that the primary xylem retains more primitive anatomical features than the secondary xylem, especially in the tracheary elements, is explained by Bailey's

(1944) refugium theory, which Carlquist (1962) used in developing his theory of paedomorphic wood. In Bailey's refugium theory, vessels evolved and became more specialized in the secondary xylem first. Evolution and specialization then proceeded to the late primary xylem (the metaxylem, which matures after elongation of the primary plant body is completed), and then to the early primary xylem (the protoxylem, which matures in actively elongating tissues of the primary plant body). As a result of these general evolutionary trends, the secondary xylem tends to have more specialized features than the primary xylem (Bailey 1944; Esau 1977).

Carlquist (1962) found many of the less specialized anatomical features that tend to be retained in the primary xylem were expressed in the secondary xylem in the herbs, woody herbs, rosette trees and shrubs, and stem succulents included in his initial study of paedomorphosis in dicotyledonous woods. Later studies confirmed the various anatomical features associated with paedomorphosis in secondary xylem uncovered in his earlier work (Carlquist 1962, 1974, 1983, 1989, 1997, 2001, 2003; Lens et al. 2005 a., 2005 b.). The anatomical characteristics include decreasing or stable vessel element length as the secondary xylem ages, and vessel element scalariform lateral wall pitting. Vessel elements often have simple perforation plates in paedomorphic wood, but there are species with scalariform perforation plates. Secondary xylem containing either wide, thin walled fibers, or parenchyma cells instead, is another characteristic common to paedomorphic woods. However, the most common characteristic related to xylary fibers is the prominence of libriform fibers (tracheids are not usually found in paedomorphic woods). Last, many paedomorphic woods are either rayless, or have rays that consist of

mostly upright ray cells (or their morphological equivalent, square ray cells) (Carlquist 1962, 2001).

Many paedomorphic woods do not have all of these characteristics. Many of the characteristics associated with paedomorphosis can be expressed independently of each other, and many species may have these characteristics to a limited degree. One or two features associated with paedomorphic wood can occur in xylem that shows a typical pattern otherwise (Carlquist 1962). In addition, it is important to note that not all of the features associated with paedomorphosis in secondary xylem, such as libriform fibers and simple perforation plates in vessel elements, are necessarily less specialized (i.e. primitive) (Carlquist 1962, 2001).

Perhaps the most important characteristic that indicates paedomorphic wood is vessel element length in the secondary xylem. Typically paedomorphic wood has either vessel elements that decrease in length over time as the secondary xylem grows (as typified by *Talinum guadalupense*), or it has vessel elements that initially decrease in length during very early growth of the secondary xylem, but then maintains roughly the same element length over the remainder of the xylem life cycle (as typified by *Macropiper excelsum*) (Carlquist 1962, 2001). If vessel element length as seen in these two plants were graphed [the graphed curves are referred to as age-on-length curves (Carlquist 1962)], the former case would show a negatively sloped curve for vessel element length in the secondary xylem, while the latter case would show a nearly flat curve for vessel element length in the secondary xylem (Carlquist 1962).

The two paedomorphic patterns of vessel element length contrast with the typical pattern seen in a "normal" woody dicotyledon such as *Eriobotrya japonica*. In the secondary xylem of dicotyledons like *Eriobatrya japonica*, the earliest formed vessel elements continue the decrease in length initiated earlier in the primary xylem. However, at some point fairly early in the secondary xylem, the vessel elements begin to increase in length. Later as the secondary xylem matures, vessel element length levels off, and may eventually even decline somewhat. In comparison, the vessel element age-on-length curve of a plant with paedomorphic wood like *Talinum guadalupense* continues the trend of decreasing element length begun in the primary xylem indefinitely. The age-on-length curve for vessel elements found in a plant with paedomorphic wood like *Macropiper excelsum* continues the trend of ever decreasing element length begun in the primary xylem very briefly, but then the lengths stabilize to relatively unchanged vessel element length in the older secondary xylem (Carlqist 1962, 2001). This results in a relatively flat curve. The paedomorphic age-on-length curves are equivalent to the descending portion (which is found mostly in the primary xylem) of the "normal" vessel element age-on-length curve for a typical woody dicotyledons. A graph illustrating these age-on-length curves can be seen in Figure 7. (Carlquist 1962).

Flat age-on-length curves indicating paedomorphosis are found for vessel elements in several woody annuals of the Asteraceae, such as *Dicoria canescens*, *Gnaphalium californicum*, and *Helianthus annus*. They are also found in stem succulents like *Cereus gigantea* (Cactaceae) and *Senecio praecox* (Asteraceae), rosette trees like

Carica candamarcensis (Caricaceae), and woody herbs like *Sonchus leptocephalus* (Asteraceae) (Carlquist 1962).

Negative sloped age-on-length vessel element curves indicating paedomorphosis are also found in several woody annuals in Asteraceae, including *Ambrosia hispada*, *Blepharizonia plumose*, *Cirsium californicum*, and *Madia sativa*. Species such as the stem succulents *Begonia coccinea* (Begoniaceae) and *Brighamia insignis* (Lobeliaceae), the woody herb *Neurolaena lobata* (Asteraceae), and the rosette tree *Scaevola kauaiensis* (Goodeniaceae) are other examples of plants with negative age-on-length curves (Carlquist 1962). Other studies document *Impatiens arguta*, *Impatiens niamniamensis* (Lens et al. 2005 b.), *Corema*, and *Empetrum* (Carlquist 1989) as examples of paedomorphic wood containing shorter vessel elements as the xylem grows.

The mechanics behind the failure of vessel elements to lengthen over time in paedomorphic wood are straight forward. In a typical woody dicotyledon, the transverse divisions in fusiform cambial initials increase in the primary xylem over time so that vessel element length decreases gradually. In the transition from primary to secondary xylem the transverse divisions are reduced, and intrusive growth in the fusiform cambial initials elongate the cells, so that the vessel elements become longer. Eventually transverse divisions in fusiform cambial initials increase and intrusive growth becomes more limited, so that vessel element length plateaus, then drops slightly. However, in paedomorphic wood the transverse divisions in the fusiform cambial initials continue, together with little cell elongation. If this is maintained indefinitely, then a plot of vessel element measurements gives a negatively sloped curve. If it continues for a shorter time,

then a plot of vessel element measurements produces a relatively level curve after the initial brief negative slope (Carlquist 2001).

Whether the reduction in length (or the maintenance of the same length) of vessel elements in paedomorphic woods offers any evolutionary advantage is unknown. It may simply be that since many paedomorphic woods are found in forms like short rosette shrubs which have thick parenchyma-filled stems, the increased mechanical strength offered by longer vessel elements (which form stronger vessels than short elements) is less important, and is not selected for (Carlquist 2001).

Scalariform (or scalariform-transitional) lateral wall pitting between vessels, and between vessels and parenchyma cells, is another anatomical feature common to many plants with paedomorphic secondary xylem. Normally this pattern of lateral wall pitting is not found in vessels in families with specialized features, unless there is paedomorphosis (Carlquist 1962, 2001). *Begonia coccinea*, *Cereus gigantea*, *Carica candamarcensis*, *Chimantaea mirabilis*, *Espeletia hartwegiana*, *Phoenicoseris regia*, *Senecio praecox* (Carlquist 1962), *Pentaphragma decurrens* (Carlquist 1997), and *Impatiens niamniamensis* (Lens et al. 2005 b.) are examples of plants with paedomorphic woods that have vessel element scalariform wall pitting. Often wide pit apertures (the pits are horizontally widened) are also found in the vessels with scalariform pitting (Carlquist 1962, 2001). Except for *Chimantaea mirabilis*, all of the previously mentioned examples of plants with vessel scalariform lateral wall pitting also have large pit apertures (Carlquist 1962, 1997; Lens et al. 2005 b.). Vessels with wider than normal pit apertures have less cell wall strength, but are common in highly parenchymatized woods. Since the

parenchyma in these plants provides mechanical strength, it may be that there is no negative selection against this paedomorphic characteristic (Carlquist 2001).

Apparently Carlquist (2001) modified his characterization of this paedomorphic feature somewhat in his later writings, and included a feature he described as vessel pseudoscalariform lateral wall pitting, in addition to scalariform lateral wall pitting. Scalariform lateral wall pitting is seen in vessels that have flattened sides (facets), and the lateral length of the pits fit the width of the flattened side of the vessel. In contrast, in pseudoscalariform lateral wall pitting there are pits shorter than the flattened vessel sides, as well as longer pitting which can extend around the circumference of the vessel (rather than remaining restricted to one flattened side, as seen in scalariform lateral wall pitting) (Carlquist 2001).

In addition to scalariform and pseudoscalariform lateral wall pitting, most plants with paedomorphic wood have vessels with simple perforation plates (Carlquist 2001). However, there are exceptions. In the families Campanulaceae, Pentaphragmataceae, Valerianaceae, and Asteraceae scalariform perforation plates which are retained relictually in vessel elements of the primary xylem may be expressed occasionally in the earlier formed secondary xylem in a few genera. In the genera where they are found in the secondary xylem, this may indicate paedomorphosis (Carlquist 1983, 1997). For example, scalariform perforation plates are found in the vessel elements of the three species of *Pentaphragma*, all of which are considered to have paedomorphic wood (Carlquist 1997). In Campanulaceae and Pentaphragmataceae the plants which have

scalariform perforation plates are mesophytes living in moist forest understories, so this characteristic may persist because it is not selected against (Carlquist 1983, 1997).

In many plants with paedomorphic secondary xylem, parenchyma cells will be extremely abundant, or will even be the only axial component of the secondary xylem apart from vessels. This is often just a continuation of the primary xylem structure in these plants (Carlquist 2001). In contrast, many typical woody dicotyledons have primary xylem made up largely of parenchyma, but a secondary xylem with mostly fibers or tracheids in addition to vessels (Carlquist 1962). Abundant axial parenchyma is found in paedomorphic wood in plants like *Impatiens niamniamensis* (Lens et al. 2005 b.), *Carica candamarcensis*, *Wunderlichia mirabilis*, *Scaevola kauaiensis*, *Brighamia insignis*, and *Talinum guadalupense* (Carlquist 1962). Since many plants with paedomorphic wood have rosette shrub or tree forms, parenchyma turgor may provide sufficient mechanical strength, thus lessening the value for fibrous tissue (Carlquist 2001).

However, thin walled, relatively wide libriform fibers are commonly found in other paedomorphic wood (Carlquist 1962, 2001). Fiber tracheids are found in a few others (Carlquist 2001). Tracheids are not usually found in paedomorphic wood (Carlquist 2001). *Impatiens arguta* (Lens et al. 2005 b.), *Begonia coccinea*, *Cereus gigantea*, *Senecio praecox*, and *Sonchus leptocephalus* (Carlquist 1962) are examples of plants with paedomorphic secondary xylem containing thin walled, wide libriform fibers. Fiber tracheids are found in the branching canes of *Chloranthus spicatus*, a species with a degree of paedomorphosis (Carlquist 1992), and also in the rayless, paedomorphic wood of *Pentaphragma* (Carlquist 1997).

Another anatomical feature seen in plants with paedomorphic secondary xylem is the presence of rays in the secondary xylem with exclusively, or at least predominantly, upright (or square) ray parenchyma cells, with few or no procumbent ray parenchyma cells (Carlquist 1962, 1970, 1983, 1989, 2001). This is in contrast to most typical woody dicotyledons, where procumbent ray cells are generally more common (Mauseth 1988). In some species a few rows of procumbent cells may be in the middle portion of larger rays (Carlquist 1962). Frequently paedomorphic rays are high and wide (Carlquist 1962; Lens et al. 2005 b.).

In paedomorphic wood the increased number of upright ray cells results from a decrease in the transverse divisions of ray initials, and lasts for the life of the plant (Carlquist 2001). The phenomenon is seen in *Impatiens niameniamensis* (Lens et al. 2005 b.), *Vernonia salvinae*, *Wunderlichia mirabilis*, *Brighamia insignis*, *Delissea undulate*, *Lobelia gibberoa*, *Trematolobelia macrostachys* (Carlquist 1962), and some members of Polygonaceae such as *Antigonon leptopus* (Carlquist 2003).

An absence of transverse divisions in ray initials leads to raylessness in some paedomorphic woods. This can be for the life of the plant, as seen in *Impatiens arguta* (Lens et al. 2005 b.), and also in species of *Plantago*, *Viola*, *Stylidium*, *Besleria*, or *Aeonium* (Carlquist 2001). In other cases the occurrence of transverse divisions in ray initials is only delayed, and the plant eventually develops rays as the secondary xylem grows. This happens in *Cyrtandra* (Carlquist 2001).

In rayless plants with paedomorphic secondary xylem like *Plantago princeps*, some fiber tracheids appear to be derived from areas of the cambium that would normally

produce rays (Carlquist 1997). The substitution of fiber tracheids for ray cells in rayless species in herbaceous groups may provide additional structural support for stems. Thus, raylessness may be positively selected for in some paedomorphically woody species which have relatively shortlived stems (Carlquist 2001).

As mentioned earlier, plants may express the paedomorphic features just discussed-paedomorphic age-on-length curves for vessel element length, vessel element scalariform lateral wall pitting with simple perforation plates (or occasionally scalariform perforation plates), thin walled libriform fibers, parenchymatous stems, rays with predominantly upright (or square) ray parenchyma cells, or raylessness-independently of each characteristic (descriptions of these features are summarized in Table 1). The number of these anatomical characteristics expressed in the secondary xylem reflects the degree of paedomorphosis present in the given species or genera. For example, there may be only a few paedomorphic characteristics present, as in *Pentaphragma*. *Pentaphragma* has rayless wood, and scalariform perforation plates and wide apertures on the lateral wall pitting of its vessels (Carlquist 1997). In other cases, such as *Foeniculum vulgare*, there may be several paedomorphic characteristics present in the secondary xylem. A negative sloped age-on-length curve for vessel element length, vessel element lateral wall pitting, wide apertures on the vessel pits, mostly erect ray cells, and thin walled, wide libriform fibers are found in *Foeniculum vulgare* (Carlquist 1962). Both of these have a degree of paedomorphosis expressed in the secondary xylem, but obviously *Foeniculum vulgare* has a greater degree of paedomorphosis.

An important point to make about paedomorphic anatomical characteristics is that they indicate the existence of paedomorphic wood only. By themselves, the characteristics should not be used to make decisions about whether or not the ancestry of a plant is herbaceous (i.e. it has secondary woodiness) or woody, although they may provide some circumstantial evidence. They cannot be conclusive by themselves because paedomorphosis can exist in plants with either woody or herbaceous ancestors (Carlquist 1962). As a result, phylogenetic relationships should be determined on evidence other than solely wood anatomical evidence, such as molecular data (Carlquist 1962, 2001). Molecular data, often sequences from non-coding regions, are useful because it is assumed to be independent of morphological variation, and avoids the problem of homoplasy in resolving phylogenetic relationships (Jorgensen and Olesen 2001).

Not all aspects of Carlquist's (1962) theory of paedomorphosis have been supported in the literature. Carlquist based part of his theory on Bailey's refugium theory. Bailey (1944) proposed that advanced features (which means features adapted for maximum conductance) of tracheary elements first evolved in the secondary xylem, and then evolved in the primary xylem. This evolutionary path explains why primary xylem tends to retain more primitive (a less optimal structure for conductance) tracheary elements than the secondary xylem of the same plant (Bailey 1944; Carlquist 1962; Mauseth 1988). In Carlquist's (1962) theory of paedomorphosis, anatomical features that are usually seen in the primary xylem of typical woody dicotyledons are extended into the secondary xylem of paedomorphic woods. Therefore in paedomorphic wood, at least

some of the anatomical features of the secondary xylem will be primitive (Carlquist 1962).

However, Bailey's (1944) ideas on the levels of evolution for tracheary elements within a single plant are disputed today. Many no longer believe certain anatomical features of tracheary elements are necessarily superior (or primitive or advanced) based only on the criterion of maximum conductance, and exist in a given type of tissue solely because of a linear primitive to advanced evolutionary pathway. Instead, it is now thought that the different types of tracheary elements have evolved in response to the environmental and physical demands put on the cells of the particular vascular tissue. Very young plants with only primary xylem may grow under different conditions, and may have different physiological needs than older plants of the same species that have developed secondary xylem. Since different demands are put upon their vascular systems, the tracheary elements must assume different structural forms, and have evolved to meet those demands. For example, younger plants may be shorter, so that root pressure is sufficient to move water and minerals up through the xylem. In this case tracheids and narrower vessels may suffice. Older plants that are larger, taller, and dependent on cohesion-tension to move water may need wider vessels to meet their water transportation needs and longer vessels to provide additional structural strength (Mauseth 1988). There are, of course, tall plants like the conifers which do not rely on wide vessels to meet their water requirements. Instead, conifers use tracheids as water conduits. However, since conifers are often found in habitats where resources are limited, the lower

resource requirement of their xylem may offer an advantage that outweighs conductivity (Hacke et al. 2001).

Mabberly (1974) disagreed with Carlquist's (1962) use of the negative sloped age-on-length and flat age-on-length curves for vessel elements as a partial basis for his theory of paedomorphosis. Mabberly noted that plants like *Talinum guadalupense* (which produced a negative sloped age-on-length curve for vessel elements as the xylem aged) and *Macropiper excelsum* (which produced the flat age-on-length curve) are pachycauls, which means in part that they have piths which become progressively wider higher in the stem. The third plant which Carlquist (1962) used in his comparison of vessel element age-on-length curves, *Eriobotrya japonica*, has a different growth form with a small pith of relatively stable width along the length of its stem. Mabberly argued that even if the vessel element measurements that produced the curves were taken at the same height, they were not taken from geometrically equivalent parts of the plants. This would necessarily produce different types of curves due to the differences in pith radius in the pachycaul plants (*Macropiper excelsum* and *Talinum guadalupense*) and the leptocaul (denotes a typical tree or shrub) *Eriobatrya japonica* (Mabberly 1974).

Basic geometry illustrates in part why different curves would be produced by the differences in pith radii. The increase in the amount of the amount of xylem is dependent on two factors: the number of cambial initials available for division and the rate of cell division. The number of cambial initials available for division is given by the formula $(2\pi r)/x$, where x = the width of cambial initials, r = the radius of the pith and secondary xylem, and $2\pi r$ = the perimeter where the vascular cambium is located. As is apparent

from the formula, the shorter the radius, the smaller the perimeter and the fewer the cambial initial cells available for division (Mabberly 1974).

With a shorter radius in a plant with a small pith like *Eriobatrya japonica*, fewer cambial initials are available for division along the perimeter of the cambium, so the rate of cambial cell division becomes relatively more important as a factor in increasing the amount of xylem. In this situation, if the rate of multiplicative radial anticlinal cambial initial division (which increases the number of initials by division perpendicular to the radius of an initial) cannot keep up with the additive divisions that add cells to the secondary xylem and phloem, then apical intrusive growth of the initials (they divide by pseudotransverse division, then elongate, and the new cells lie side by side) (Esau 1977) becomes more important in increasing the number of initials.

Intrusive growth increases the number of initials available for division. An initial which lies above another initial may elongate so that part of it wedges between the lower neighboring initial and its neighbor. Transverse cell division in the elongated cell then results in two cells, where there originally was only one initial. This also helps to increase the perimeter or circumference of the vascular cambium. In addition, the increased role of apical intrusive growth of the initials while the radius is small results in longer vessel elements (Mabberly 1974).

Over time as the radius increases (with an accompanying increase in the perimeter of the cambium) there are more cambial initials available for radial anticlinal division, so the need for intrusive growth in the initials (which produces longer vessel elements) to increase the number of initials is reduced. As a result if vessel element length is plotted,

there is a steep rise in vessel element length in earlier formed secondary xylem, then a reduction, with a plateau eventually reached (Mabberly 1977).

At points above the base of the plant, pachycaul plants like *Macropiper excelsum* and *Talinum guadalupense* have piths that become increasingly wider. The greater radius that results means that there are more cambial initial cells available for radial anticlinal division, so it is easier for multiplicative cambial initial division to keep up with additive cell divisions that increase the xylem. Therefore, there is less need for apical intrusive growth to increase the number of initials, and vessel element lengths do not increase. Since these plants start with a wider pith, a plot of their vessel element lengths tend to look like the latter part of the age-on-length curves plotted from the vessel elements contained in typical woody dicotyledons. However, if vessel elements were measured at the base of the pachycaul plants, then the age-on-length curves of both the typical woody dicotyledons and pachycauls would show the same pattern, since both plant types have a small pith at the base of the stem (Mabberly 1974).

Since the age-on-length vessel element length curves produced by the pachycaul plants in Carlquist's (1962) study can be explained by the wider pith at the location where the measurements were taken rather than by paedomorphosis, Mabberly (1974) argues that the theory of paedomorphosis is not supported by the curves. Measurements of vessel elements of *Macropiper excelsum* and *Talinum guadaloupense* should be taken at the base of the plants for a valid comparison between their age-on-length curves and the curve produced by a typical woody dicotyledon like *Eriobatraya japonica*. Without a

comparison of vessel element measurements taken at geometrically equivalent locations, the theory is unproven (Mabberly 1974).

Insular Woodiness

Many plants that have paedomorphic wood are found on islands or equatorial highlands. *Sonchus leptocephalus* (Canary Islands), *Brighamia insignis* (Hawaiian Islands), *Delissea undulata* (Hawaiian Islands), *Lobelia gibberoa* (Hawaiian Islands), *Scaevola kauaiensis* (Southern Polynesian Islands), *Talinum guadalupense* (California offshore islands), *Chimantea mirabilis* (Guiana highlands), and *Carica candamarcensis* (the Andes of northwestern South America) are examples of species that have paedomorphic secondary xylem, and are considered to have insular woodiness as well (Carlquist 1962, 1974). Insular woodiness refers to the increased woodiness of insular plants from predominantly herbaceous groups. In this context, insular can mean oceanic islands, continental islands, or mountainous areas near the equator. Woodiness refers to the amount of secondary xylem in comparison to relatives on the mainland or the lower elevations in the area (Carlquist 1970, 1974).

Oceanic islands differ from continental islands in geologic origin, age, and their source of biota. Oceanic islands such as the Hawaiian Islands and the Canary Islands are of volcanic origin, and are geologically much younger than continental islands. Often they are more isolated than continental islands: but there are exceptions such as the Canaries of Macaronesia, which are only 322 km from continental Africa (Givnish 1998). Their entire flora and fauna are necessarily derived from colonization from mainland areas (Hubbell 1968). Continental islands like New Zealand, Madagascar, and the

California Islands (except perhaps Guadalupe) were once attached to continents, but were separated from them by rising sea levels (Hubbell 1968; Thorne 1969). As a result of their geologic origins, they are typically much older, and are frequently less isolated than oceanic islands (Thorne 1969). In addition, during the time they were joined to the continents, they shared the same flora and fauna with the continent. As a result, at least part of their biota is derived from the continent from that earlier period, as well as from later colonization (Hubbell 1968).

Equatorial highlands share certain characteristics with islands such as relatively uniform yearly climates, and in a sense they form "islands" of isolated areas that contain flora and fauna that are distinctly different from that of the surrounding area. Examples of equatorial highlands include the New Guinea highlands, the highlands of Colombia and Venezuela, and Mt. Kenya in eastern Africa (Carlquist 1974).

Plants with insular woodiness can have a wide variety of growth forms. Some are rosette herbs which have undergone extended vegetative growth over time. The extended growth period produces elongated stems which flower eventually. *Wilkesia* (Hawaii), *Echium pininana* (Canary Islands), and *Senecio keniodendron* (Kenyan plateau) all have insular woodiness and the rosette growth form. Sometimes rosette plants develop lateral branches at the base, which converts the monocarpic [flowering and bearing fruit once and then dying (Harris and Harris 2003)] rosette plant into a shrub. The shrub-like growth form of *Stephanomeria blairii* (San Clemente Island) is an example of this (Carlquist 1974).

Some plants with insular woodiness grow lateral branches from nodes beneath the inflorescences to produce a candelabrum growth form like that seen in *Echium* (Macaronesia) and *Euphorbia candelabrum* (Kenyan plateau). Axillary inflorescences [those positioned between the axis of the stem and another part like a leaf (Harris and Harris 2003)] whose axis continues to grow indefinitely may occur in some woody growth forms like *Plantago* (San Fernandez, Hawaii) (Carlquist 1974). Some already shrubby genera like *Sarcopygme* (Samoa) have increased in height on islands (Carlquist 1974).

Other plants with insular woodiness may be arborescent with erect, bloated trunks and thick main branches (the "bottle tree" pachycaul habit) like *Dendrosicyos socotrana* (Socotra Island off the coast of Yemen) (Olson 2003). Other genera have become woody and tree-like in very dry conditions, such as *Sida eggersii* (West Indian Islands) (Carlquist 1974). Other examples of plants with insular woodiness that have tree-like growth forms are *Senecio vaccinioides* and *Hesperomeles ferruginea* (the paramos highlands of the Andes) (Carlquist 1974).

There are two broad hypotheses for the origin of insular woodiness. One hypothesis is that plants with insular woodiness are relicts: they are descended from woody continental ancestors which are now extinct (Carlquist 1974; Mort et al. 2002). In some cases where the island plants are minimally woody and are nearly herbaceous, they evolved to lose some of their woodiness (Carlquist 1974). The relictual origin has been hypothesized for woody taxa in Macaronesia (the four archipelagos of the Azores, Madeira, Canary Islands, and Cape Verde in the North Atlantic Ocean). In Macaronesia

woody taxa are descended from woody continental ancestors which were once abundant, but were driven to extinction in Europe during the Pleistocene glaciation and in Africa by desertification. Insular woodiness in this scenario is an evolutionarily ancestral (or primitive) character state, or plesiomorphic (Barber et al. 2002; Goodson et al. 2006).

The second hypothesis is that plants with insular woodiness recently evolved from herbaceous ancestors on the continent (mainland) in response to various environmental factors on the islands (Carlquist 1974; Barber et al. 2002; Mort et al. 2002; Goodson et al. 2006). In this scenario insular woodiness is an evolutionarily derived character state, or apomorphy. Insular woodiness with this origin could also be called a form of secondary woodiness. Research to date supports the first hypothesis of relictual insular woodiness in some cases. However, the second hypothesis of derived insular woodiness is supported more frequently in specific island plant lineages (Mort et al. 2002). In addition, the second hypothesis has led to several alternative, detailed hypotheses (almost a subset) in attempt to explain why insular woodiness occurs in this manner.

Providing support for either hypothesis for the origin of insular woodiness can be problematical. Many island plants have diverged so much from mainland ancestors that it is difficult to discern patterns of relationships and evolution (Givnish 1998; Kim et al. 1996). Typical problems involve determining the source of the original island colonizers, when the colonization took place, the number of introductions which led to colonization, and the ancestors of the island genera (Givnish 1998). Although morphological data has been used to address these problems, sometimes it is difficult to tell whether a shared character state is due to common ancestry, or due to convergent evolution (where two

unrelated organisms acquire the same character state from different ancestors) (Givnish 1998). Using molecular sequences as a basic for phylogenetic analysis has increasingly been used, both in conjunction with morphological data and by itself, to determine the origin of insular woodiness in various genera. However, even molecular data is subject to interpretation, and phylogenetic analyses developed from it do not always present clear cut, unambiguous answers. This, together with differing interpretations of morphological data, can result in different analyses.

An example of how similar data can be interpreted differently can be seen in two phylogenetic studies based on chloroplast DNA (cpDNA) and internal transcribed spacer (ITS) sequences. One study looked at *Descurainia*, a genus of perennial woody shrubs found in the Canary Islands (Goodson et al. 2006), and the other study examined *Sideritis*, which is composed of suffrutescent (having a woody base with an herbaceous upper stem) perennials, rosette plants, and arborescent shrubs in Macaronesia, and suffrutescent annuals and perennials on the mainland (Barber et al. 2002). In the phylogeny developed for *Descurainia*, *Descurainia tanacetifolia*, which is the closest mainland relative, is basal to the insular species of *Descurainia*. It is also suffrutescent. As a result, it was extrapolated that woodiness may have been present in the continental ancestors, and that insular woodiness in this case is relictual (Goodson et al 2006). In contrast, with *Sideritis* the phylogeny indicated an increase in woodiness among the insular members, although the ancestral habit was not firmly identified. The mainland species ranged from herbaceous annuals to suffrutescent perennials, while the insular

species ranged from suffrutescent herbs to large woody herbs. The range of habit was interpreted to indicate woodiness is derived in *Sideritis* (Barber et al. 2002).

Other studies have supported the hypothesis that insular woodiness is relictual in some genera. A phyologenetic analysis of the genus *Pericallis*, which is composed of woody subshrubs and herbaceous perennials in Macaronesia, used morphological data, molecular data, and a combined data set. Although not conclusive, the analysis provided evidence that the ancestral state for *Pericallis* is woody, not herbaceous (Swenson and Manns 2003). Firm support for the relictual origin of insular woodiness has been found for *Lactoris fernandeziana* (Juan Fernandez Islands) based on anatomical, developmental, and molecular data (Mort et al. 2002; Fuentes-Aguilar et al. 2002). A molecular study using two chloroplast genes supports relictual insular woodiness for *Plocama pendula* as well (Canary Islands) (Bremer 1996; Andersson and Rova 1999; Fuentes-Aguilar et al. 2002; Goodson et al. 2006). In addition, *Tolpis* (Macaronesia), which is a mostly woody genus, possesses insular woodiness which originated relictually (Moore et al. 2002). The molecular based phylogeny for *Dendrosenecio* (east African highlands) suggests its ancestors were at least semiwoody (Knox and Palmer 1995).

The hypothesis that insular woodiness is derived from herbaceous ancestors from the mainland is supported for a number of genera in the literature. Molecular based analyses support derivation of woodiness from herbaceous ancestors for *Sonchus* in Macaronesia (Kim et al. 1996), *Lavatera* in the Canary Islands (Fuentes-Aguilar 2002), *Echium* in Macaronesia (Bohle et al. 1996), *Aichryson* (Fairfield et al. 2004; Mort et al. 2002) in Macaronesia, *Aeonium* (Mort et al. 2002) in Macaronesia, *Greenovia* in

Macaronesia (Mort et al. 2002), *Monanthes* in Macaronesia (Mort et al. 2002), and the Hawaiian silverswords (*Argyroxiphium*, *Wilkesia*, *Dubautia*) (Baldwin 1998).

There are also several more detailed hypotheses that attempt to explain why plants with insular woodiness may have evolved from mainland herbaceous ancestors, rather than being relicts. These hypotheses can be seen as a subset of the broader hypothesis. Two of the older hypotheses are Darwin's (1859) competition hypothesis and Wallace's (1878) longevity hypothesis.

When Darwin (1859) visited the Galapagos Islands during his famous voyage on the *Beagle*, he noticed the tree sunflowers (*Scalesia*) growing there had insular woodiness. He hypothesized that while trees were unlikely to reach the islands [he was unsure why this was], herbaceous plants would be successful. Herbaceous plants could then gain competitive advantage by growing taller than other plants. This would lead to selective pressure for increased woodiness in shrubs, which would eventually lead to arborescence (Darwin 1859; Givnish 1998).

Since seeds or spores of land plants have to be capable of dispersing over long distances to reach and colonize islands (Givnish 1998), some have speculated that the large size and lower viability of seeds from continental trees preclude those trees from colonizing islands (Carlquist 1974). In addition, one argument supporting Darwin's hypothesis is that the open or partially open habitats found on islands early in their colonization lend themselves to colonization by sun adapted herbs. Open habitats are likely to be visited by birds releasing seeds via endozoochory (dispersal of seeds from fleshy fruits in the digestive tract) or ectozoochory (dispersal of seeds stuck to feathers or

skin) from similar environments on the mainland. In the island environment there would be a gradual increase in plant coverage away from the open habitat which would encourage evolution of increased woodiness leading to increased stature (Givnish 1998). The increase in woodiness which accompanies the change in habitats from open habitat to forest habitats, especially wet forest, is sometimes referred to as an ecological shift (Carlquist 1974). In many cases the woodier herbs are understory plants, or occupy more open forest areas, or else take on shrubby forms in scrub areas (Carlquist 1974). The competition hypothesis is also supported by models and quantitative measurements in recent studies (Givnish 1982, 1998; Tilman 1988).

Wallace (1878) hypothesized that woodiness would evolve in herbaceous island colonists as a way to extend their life span. With greater longevity, there would be more flowers over time, which would allow a greater chance for cross-pollination by insect pollinators, which are not common on islands (Wallace 1878; Carlquist 1974; Givnish 1998; Jorgensen and Olesen 2001). However, this hypothesis not considered to be well supported. First, many plants can self-pollinate. In addition, on islands insect pollinators tend to be either very abundant (since they have no competition), else they are absent entirely (they have not dispersed to the island) (Carlquist 1974; Givnish 1998).

Wallace's (1878) longevity hypothesis was expanded in a molecular study of the genus *Echium*, mostly woody perennials found in Macaronesia (Bohle et al. 1996). Since *Echium* is insect pollinated, and its environment would not contain many insects initially, increased woodiness would provide the same opportunities for increasing pollination as those cited by Wallace. In addition, there is inbreeding in the herbaceous members of

Echium found on the mainland high enough to cause inbreeding depression in new island populations. Selection against inbreeding would lead to the large inflorescences found on island *Echium*, which could help attract pollinators, and the woody habit necessary to produce the inflorescences. Thus the woody habit of *Echium* found in Macaronesia has been hypothesized to help prevent inbreeding depression in geographically isolated founding populations (Bohle et al. 1996).

The climatic moderation found on islands as well as equatorial highlands is another hypothesis used to explain the evolution of woodiness by insular plants from herbaceous ancestors (Carlquist 1974; Jorgensen and Olesen 2001). The climate is moderated in the sense of either remaining uniform throughout the year, or else having any climatic extremes minimized by oceanic influences. Climatic moderation includes components like moderate annual temperatures, adequate and uniform rainfall, and high humidity. Moderation in these components provides a release from seasonality for the island plants, so that continuous growth is possible. Continuous growth allows a root system that can support more leaf growth for additional photosynthesis, and leads to increased woodiness which results in greater arborescence (Carlquist 1974).

Moderate annual temperatures in island environments are mean temperature ranges like 10-25°C (found on Hawaii, Fiji, and New Caledonia) or 15-20°C (found on Juan Fernandez and St. Helena). Moderate temperature ranges promote a long growing season that is favorable for promoting woodiness and arborescence in herbaceous plants (Carlquist 1974).

Annual rainfall in the amount of at least 1000 mm is found on islands such as Juan Fernandez and St. Helena at sea level. In equatorial highland areas cloud cover can also make moisture available in drier alpine areas. In association with rainfall, high humidity resulting from the nearby ocean can envelope small island areas to provide insulation against temperature fluctuations. High humidity also helps reduce transpiration as well as evaporation from the soil. Humidity in some locations in Hawaii averages 81% annually, while in the Canary Islands it may average 75% annually (Carlquist 1974).

Although climatic moderation permits increased woodiness and arborescence, some argue that the cost of growth in height cannot be ignored (Givnish 1998). Taller plants incur energy costs because they allocate more resources to support tissues, which leaves less energy available for additional growth and reproduction. The only way to overcome this is to increase photosynthesis by overtopping their competition (Givnish 1998).

The absence of herbivores on islands is another hypothesis used to explain the increased woodiness evolved by herbaceous plants on islands. Most islands have not had large herbivores historically, which has allowed herbaceous plants to complete their life cycles, and to exploit climatic moderation. Thus, rather than being consumed by herbivores, the plants enjoy year-round growth, which has led to increased woodiness and woody growth forms eventually (Carlquist 1974; Jorgensen and Olesen 2001). On some islands where herbivores have been introduced, predatory pressure has prevented the reproduction of woody plants in areas accessible to the herbivores. On these islands, only annuals and woody plants on inaccessible cliffs tend to survive heavy predation. On other

islands such as Hawaii, long-term herbivory pressure threatens the survival of some woody herbaceous plants (Carlquist 1974).

Secondary Woodiness

Like paedomorphosis and insular woodiness, the term secondary woodiness is not always used consistently in the literature. Even the concepts of "woody" or "woodiness," which are used much more often in the literature, are used ambiguously. Although wood is generally understood to mean the secondary xylem of gymnosperms and dicotyledons (Esau 1977), when applied to growth forms woodiness can mean a variety of different things. For instance, a plant may have a woody stem, a woody root system, or both (Isnard et al. 2003). Woodiness in growth forms can also exist in varying degrees, from a small amount to a very substantial amount. A sufrutescent stem like that found in *Descaurainia tanacetifolia* has secondary xylem only at its base, while the remainder of the stem is herbaceous. At the other extreme, much of the tissue in plants like *Acer rubrum* is secondary xylem. Thus, whether a particular growth form is woody may be open to interpretation.

Secondary woodiness is not discussed frequently in the literature. However, it is best described as the evolution of a moderate amount of secondary xylem in members of largely herbaceous plant groups from herbaceous ancestors (Carlquist 1974, 1992; Lens et al. 2005 a.; Lens et al. 2005 b.). The term itself suggests that woodiness was lost within a group, and then reappeared during later evolution (Isnard et al. 2003). It also implies that primary woodiness, a term rarely used in the literature, exists. Primary woodiness

may be inferred to mean woodiness derived from a woody ancestor (i.e. woodiness is plesiomorphic) (Carlquist 1995, 2003).

Secondary woodiness is different from secondary growth. Secondary growth is growth in the girth, or thickness, of a plant. It produces the secondary tissues, which develop from the secondary meristems, the vascular cambium and the cork cambium (the phellogen). The vascular cambium produces the secondary phloem and the secondary xylem (wood) (Fahn 1982).

As discussed earlier, secondary woodiness is not the same thing as paedomorphosis in secondary xylem, nor is it the same as insular woodiness. In addition the existence of paedomorphosis or insular woodiness does not mean secondary woodiness necessarily caused either condition. It is true that many plants with paedomorphic wood are secondarily woody (their woodiness is derived from herbaceous ancestors), and paedomorphic anatomical characteristics frequently indicate the possibility of secondary woodiness. But herbaceous plants can evolve towards either greater herbaceousness, or greater woodiness, so it is conceivable that paedomorphic characteristics could appear in secondary xylem following either trend. As a result, the mere existence of paedomorphic characteristics is insufficient to determine if the plant has secondary woodiness. The woodiness has to be placed into a phylogenetic context, and its ancestors and relatives analyzed (Carlquist 1962).

The same holds true for plants with insular woodiness. The existence of increased woodiness in an island or equatorial highland plant cannot be used as the sole evidence supporting secondary woodiness, and insular woodiness cannot be equated with

secondary woodiness. Phylogenetic analysis must be used to resolve the origin of insular woodiness in a given genera, and it cannot be assumed to be secondary woodiness (Kim et al. 1996). In many cases insular woodiness has been found to be caused by secondary woodiness. However, this is not always true, and insular woodiness has also been found to be plesiomorphic (relictual in origin) (Mort et al. 2002).

Paedomorphosis and Secondary Woodiness in Xanthorhiza simplicissima, Coreopsis gigantea, and Mahonia bealei

Carlquist (1995 c.) states that the paedomorphic rays of *Xanthorhiza* make it possible to interpret its wood as secondarily woody. However, he did not have a stem of the maximum diameter for his study (i.e. an older stem), so he could not determine if the rays remained paedomorphic as the stem aged. In addition, members of Ranunculaceae have relatively short vessel elements (which indicates the vessels are derived from short fusiform cambial initials), especially when compared to woody families like Lardizabalaceae and Menispermaceae. If any Ranunculaceae genus were primitively woody, longer fusiform initials should be retained. In the case of *Xanthorhiza*, in general the cells found in its wood are small, so it is not clear that it possesses secondary woodiness based on fusiform initial size (Carlquist 1995 c.). Without elaboration, Judd (2002) states that woodiness is probably secondary in the Ranunculaceae family, but does not discuss *Xanthorhiza simplicissima* (Judd 2002).

There is little discussion in the literature on whether *Coreopsis gigantea* is woody, or possesses secondary woodiness. Carlquist (1974) considers it as an example of a woody herb that occurs on islands and on narrow strips of land along continental

coastal areas, with both areas having a moderated climate, and lists it in his Catalogue of Woody Herbs on Islands (Carlquist 1974). He also includes it as a member of the woody genera of the Munz flora of southern California (Carlquist 1985). Thorne (1969) also considers it as an example of insular woodiness (Thorne 1969).

Although the literature indicates that *Coreopsis gigantea* has insular woodiness, it is important not to equate its insular woodiness with secondary woodiness. As discussed previously, plants with secondary woodiness are derived from herbaceous (nonwoody) ancestors. However, there are two hypotheses for the origin of insular woodiness (in a simplified description, the ancestors were herbaceous, or the ancestors were woody). Obviously, one of the hypothesized sources of insular woodiness is in conflict with secondary woodiness.

Judd (2002) considers the genus *Berberis* to have several apomorphies (evolutionarily derived character states), including secondary woodiness (Judd et al. 2002). This is supported somewhat by Carlquist (1995), who cites two phylogenetic studies (Loconte and Estes 1989; Qiu et al. 1993) which can be interpreted that the subgenus *Mahonia* is secondarily woody (Carlquist 1995).

In the Loconte and Estes study (1989) their cladogram shows a woody ancestry for Berberidaceae, then a shift to herbaceousness, with a reversion to woodiness for the *Berberis*/*Mahonia* branch (Carlquist 1995; Loconte and Estes 1989). Loconte and Estes (1989) support their conclusion that *Berberis* and *Mahonia* are secondarily woody by citing the apomorphic characteristics (evolutionarily derived character states) of their

wood anatomy. The apomorphic character states include ring porosity, little or no wood parenchyma, and homogenous, uniseriate rays (Loconte and Estes 1989; Shen 1954).

The Figure 3. maximum-parsimony tree generated in Qiu's study (1993) places the order Papaverales basal to the order Ranunculales. The order Papaverales consists of herbaceous plants (the tree shows *Dicentra* and *Papaver* as representatives of the order). Since the order Ranuculales contains many woody genera (*Mahonia*, *Xanthorhiza*, *Cissampelos*, *Cocculus*, *Akebia*, and *Euptelea*, all woody genera, are included in the tree for Ranunculales, along with *Caltha*, which is herbaceous), the implication given by the tree is that *Mahonia* has herbaceous ancestry (Qiu et al. 1993; Carlquist 1995).

CHAPTER III

METHODS

Specimens of *Xanthorhiza simplicissima* were collected from three sites in North Carolina: Guilford County (the Randleman Lake area), Randolph County (Little River at Pisgah Covered Bridge), and Transylvania County (the Avery Creek area of Pisgah National Forest). *Mahonia bealei* specimens were collected in Guilford County, North Carolina on the campus of the University of North Carolina at Greensboro, as well as a nearby off-campus location in Greensboro. The specimens of *Coreopsis gigantea* used in the study were supplied by the University of California at Berkeley Botanical Garden. All plant material from the three species was stripped of leaves, and the stems cut into short sections of approximately 2.5 cm length. The sections were then fixed in either FAA (a formalin-acetic acid-ethyl alcohol based fixative), or Karnovsky's fixative (a glutaraldehyde based fixative).

After fixation, slides for microscope viewing were made using either stem cross-sections or macerated tissue. Stems were prepared for maceration by removing the periderm, and were then macerated using Gifford's method (Ruzin 1999). Once the maceration process was completed, the tissue samples were washed with distilled water, and transferred to 50% ethyl alcohol. Samples were then placed on glass slides, teased apart, and then stained for ten minutes with 1% safranin in 95% ethyl alcohol. Following

this, the macerated tissue was washed with 95% ethyl alcohol several times, and then washed with 100% ethyl alcohol. A final wash of Clear Rite was done, and Richard-Allan Mounting Medium was used to cover the specimens before cover slipping the slides (Ruzin 1999).

Stem cross-sections (transverse, radial, and tangential) were sectioned either by hand using a razor blade, or by sliding microtome (American Optical Company Model 860). After sectioning, a protocol similar to the maceration preparation was followed (Ruzin 1999). All sections were transferred to 50% ethyl alcohol. The sections were then stained with 1% safranin in 95% ethyl alcohol, or stained with 1% safranin, followed by staining with fast green. After staining, the sections were washed with 95% ethyl alcohol, 100% ethyl alcohol, and finally Clear Rite. They were then placed on glass slides, and covered with either Richard-Allan Mounting Medium or Karo Corn Syrup before cover slips were put over the specimens on the slides.

Another protocol for slide preparation was used which allowed slides to be used with the confocal microscope as well as the light microscope. Only specimens fixed in FAA were used, and these were hand-sectioned using a razor blade to prepare transverse, tangential, or radial sections. The sections were stained with 1% safranin in 50% ethyl alcohol for twenty to thirty minutes, and then dehydrated through a graded acetone series at fifteen to thirty minutes at each concentration (30%, 50%, 75%, 90%, 100% acetone). The sections were then rehydrated through a graded acetone series (90%, 75%, 50%, 30%, 10% acetone) to 100% distilled water using the same time intervals. After this step the sections were cleared using a graded glycerol series (25%, 50%, 75%, 100%

glycerol). Specimens were kept in each glycerol solution for at least one hour. Once the glycerol series was completed, the cleared sections were mounted on glass slides using 100% glycerol, cover slipped, and viewed (Kitin et al. 2000).

Three different light microscopes were used during the study. An Olympus CH-2 model light microscope was used to take measurements of cells and tissue, as well as for observations. Ocular units were converted from the micrometer of this microscope into μm to provide the measurements (using the 4x objective, 1 ocular unit = 20.0 μm; using the 10x objective, 1 ocular unit = 8.1 μm; using the 40x objective, 1 ocular unit = 2.0 μm). Another microscope, a Leitz Wetzlar Otholux model light microscope, was utilized occasionally. The third light microscope, a Nikon Eclipse E600 light microscope, was used together with a Diagnostic Instruments, Inc. Model 2.2.1 Revision 2 camera and SPOT Advanced Software (Windows Version 4.0.8) to take photographs of the specimens.

An Olympus FluoView™ FV500 confocal laser scanning microscope provided confocal microscopic photographs. These photographs, as well as those taken using light microscopy, were processed utilizing Adobe Photoshop CS3 Extended Version 10.0.1 software. Labeling the photographs was done using Microsoft™ Word.

Field photographs of the plant species used in the study were taken with a Nikon COOLPIX5700 model digital camera and transferred to computer using Nikon View (Windows®: Version 6.0.1) software.

Cell dimensions were measured at the longest and widest points of the cells. For vessel elements, measurements included the cell wall and caudate tips. Measurements for

vessel elements and fibers came from maceration slides. To determine the trends of vessel element lengths across the secondary xylem, the vessel element lengths were measured in radial sections. Measurements of these vessel elements were made starting with the xylem formed at the stem center, and moving towards the vascular cambium. Widths of rays were measured in tangential sections, while ray cells were measured in radial sections. Radial sections also provided insight into the extent of vessel, ray, and fiber storying. Transverse sections provided measurements for mean number of vessels per group, as well as observations about vessel restriction patterns and vessel distribution.

Statistical analyses and graphing were conducted by using Minitab 15 statistical software. Vessel element lengths taken across the width of the xylem were used in the analysis to produce two types of graphs, a graph of the data points with a Lowess smoother line fitted, and a graph of the data points with a linear regression line fitted. In addition, a linear regression analysis was done for each data set.

Lowess is the shortened form of Locally Weighted Scatterplot Smoother, which is a routine that selects a fraction of all points around each x,y data point, using data closest to the x value of that data point (on either side of the point). The routine then performs a weighted linear regression for each data point, giving points closest to the x value the greatest weight in smoothing the line. It then modifies this value by calculating new weights in order to reduce the influence of outliers. The end result is a new smoothed y value for each x value, and a curve fitted to the data (Minitab Inc. 2006).

Means and standard errors of the mean were also calculated using the statistical software. However, a word of caution about the statistical analysis is appropriate. Since

only a few plant samples were taken from a limited number of sites, it is not statistically valid to make inferences about the species populations, based on this study's results. To ensure statistically robust inferences about the population, an appropriate random sampling design would be necessary (Ramsey and Schafer 2002). While most plant anatomical studies fall short in this area, means are still calculated and used in traditional plant studies. In light of that, the means and other measurements from the thesis research may still provide useful insights.

An analysis based on published phylogenetic data was done for each of the plants used in the thesis. Each analysis consisted of two components. The first was a phylogenetic tree taken from the recent literature. Each of the trees was generated from either genetic sequences or restriction site mapping. The second component was a matrix which assigned the character state woodiness or nonwoodiness to each genus or species listed on the phylogenetic tree. The character states were determined using a survey of the literature and other sources. Information such as habit, longevity, and morphological descriptions were used to judge the likelihood of woodiness. Once each of the genera was assigned a character state (woody or nonwoody), the tree was analyzed for changes in character state throughout the branching of the tree. Following this process, the trees were analyzed to determine how the woodiness of the plants used in the thesis was derived. Woodiness could be derived from a woody ancestor (primary woodiness) or a nonwoody ancestor (secondary woodiness).

CHAPTER IV

RESULTS

Stem Anatomy and Xylem Characteristics of *Xanthorhiza simplicissima*

Overview of the Stem Anatomy

Transverse sections of *Xanthorhiza simplicissima* reveal a stem center (the pith) composed of large thin-walled parenchyma cells. Many of the parenchyma cells in the pith, as well as the periphery of the pith, contain translucent lumps of an indeterminate material. Within the pith, these cells are rounded, with some cell wall sides flattened against adjacent parenchyma cells. As they approach the xylem, the parenchyma cells become smaller, and at the base of the earliest formed vessel groups, they begin a transition to much smaller, elongated oval shaped cells (see Figure 8.). These cells gradually assume somewhat rectangular shapes between the strands of vessels which radiate from the earliest formed vessel clusters. They then form the rays of the secondary xylem. The vessels, which are rounded in transverse view, lie between the rays, and are embedded in fiber cells. The fiber cells are smaller than the ray cells, and are oval or rounded in outline, as seen in cross-section (see Figure 9.).

The xylem takes up a major proportion of the stem cross-section, especially in older stems. It essentially forms a cylinder of tissue around the pith (see Figure 10.). The most recently formed secondary xylem ends at a vascular cambial zone. Curved arches of tissue can be seen in transverse sections just beyond where the vessels of the secondary

xylem end. This tissue is composed of parenchyma cells interspersed with half moons of partially crushed phloem tissue, and the arches are frequently capped with a smaller arches of fibers. Beyond this lie the secondary cortex and the periderm of the stem (see Figure 11.).

A review of radial and tangential sections, in addition to transverse sections, shows that the secondary xylem of *Xanthorhiza simplicissima* is composed only of vessels, rays, and fibers. No axial parenchyma or fiber tracheids were found, although it is possible that some tracheids exist (see Figure 12). However, only a few examples of these were seen. In radial and tangential views, the parenchyma cells in the pith assume a somewhat oval shape which is oriented horizontally. Frequently the horizontal sides are parallel, and flattened, although other parts of the cell wall may be flattened also (see Figure 13.). At the pith/xylem interface, the parenchyma cells of the pith tend to become upright (vertically oriented) at the very margin of the pith.

Vessels

Vessels are a prominent feature of the secondary xylem. In transverse sections they appear rounded or oval in cross-section, and may have flattened sides in groups of vessels. Single vessels not appearing in groups also may have flattened sides occasionally. The vessels form single (or occasionally double) strands which radiate outwards from tangentially oriented clusters of vessels. The tangentially oriented vessel clusters consist of mostly larger diameter vessels. The radially oriented vessel strands which emerge from these clusters usually make a transition to vessels with smaller diameters. Smaller diameter vessels are especially noticeable at the very end of the

strands, which tend to end in a pronounced break just before another tangentially oriented vessel begins. Almost all of the vessels are restricted to the central part of the fasicular areas, with no contact with the rays (see Figure 14.).

In many of the transverse sections a darker stained line can be seen beneath the tangentially oriented clusters of the vessel strands. This is due to the slightly smaller cell size of the fibers and ray cells just beneath the tangentially oriented clusters of vessels. The resulting slightly higher cell density leads to differential staining, highlighting the appearance of a growth ring. In instances where there is no darker stained line, the tangentially oriented vessel clusters signal the start of a new annual growth ring. The vessels are distributed in a semi-ring porous pattern, which means they make a transition from the larger diameter vessels of the tangentially oriented clusters (the earlywood, also known as springwood) to the smaller diameter vessels of the vessel strand (the latewood, also known as summerwood). Thus, a typical growth ring is marked by a short tangential band of wider diameter vessels, consisting of at least two or three or more in contact with each other. In addition a discontinuous strand of smaller vessels, usually radial, but sometimes having diagonal sections, usually emerges from the tangential band and ends at the margin of the next growth ring (see Figure 15.).

There are a substantial number of single vessels which are separated from one another by intervening fiber cells, as well as vessel groups of two, three, or more (these are called pore multiples by wood anatomists). Extremely high numbers of vessels making contact in a group are not very common, but there were examples of groups of

ten, sixteen, or even higher seen occasionally in the data. The mean number of vessels in a group is 2.5 vessels (n = 26; SE = 0.1)

The mean length of the vessel elements in maceration preparations is 249.4 μm (n = 50; SE = 9.4), and the mean width (which includes the lateral cell walls) is 32.9 μm (n = 50; SE = 1.6). Narrow, oval shaped pits in an alternating pattern of rows are found on the secondary walls of the vessel elements. The pits are numerous and cover much of the surface of the lateral walls. The pits do not appear to be bordered, when viewed by light microscope. No examples of vessel elements with annular rings, helices, scalariform (ladder-like), or reticulate (net-like) secondary wall deposition patterns on the lateral cell walls were found. Therefore, in *Xanthorhiza simplicissima* the only part of the surface of the primary wall that is not covered by the secondary wall is the part exposed due to the pit apertures (openings) (see Figure 16.).

The vessel elements have simple perforation plates, which means the end walls of the cells have no sieve-like area or bars across the opening. Instead, they are completely open. Most of the vessel elements have ends which are slightly to fairly oblique (i.e. slanted or angled), with noticeable caudate tips (tails). In some cases the caudate tips or ends can be relatively, although not extremely, long (see Figure 17.).

Tangential sections were used to determine if vessel elements exhibited storying (when adjacent vessel elements show their end walls aligned with each other, they are considered to be storied). Except for an occasional instance where the vessel elements in adjacent vessels are aligned with each other, there is no overall vessel element storying (see Figures 18. and 19).

Vessel element lengths were measured across the width of the xylem, moving from xylem formed at the edge of the pith and moving towards the vascular cambium. A graph of these data points with a Locally Weighted Scatterplot Smoother line fitted shows a decrease in vessel element length, with more recently formed vessel elements having shorter lengths than the older vessel elements. The resulting curve has an overall negative slope (see Figure 24.). When a linear regression line is fitted to the same data points, a negatively sloped line results also (p = .002 and r^2 = 10.4%) (Figure 25.).

Rays

Rays in *Xanthorhiza simplicissima* are multiseriate (more than one cell wide in tangential view). In transverse views, they start out as two to four cells wide in the earliest formed xylem, and gradually widen as they extend as linear strands across the secondary xylem towards the vascular cambium. The ray cells tend to be somewhat rectangular in outline in transverse views (see Figure 14.).

In tangential sections, the ray cells are a mix of shapes. A few of the ray cells may be rounded, but most are oval or elongated oval shaped in outline. Some of the elongated shaped ray cells may have flattened or angled ends (see Figures 18., 20.). Many of the ray cells observed contain groups of rounded objects, or in some cases objects with flattened sides (see Figure 21.). The lateral cell walls of the ray cells have a scattering of oval shaped pits, which may exhibit an alternating pattern when there are a lot of them. The rays tend to be tall, and extend past the end of the sections. Due to this, the ray heights could not be measured. However, they have a mean width of 150.3 μm (n = 26; SE = 12.4), or 9.6 cells (n = 26; SE = 0.8). Most of the rays have a uniseriate end, where

they end in a single ray cell. However, it is common to find instances where the ends of the rays are uniseriate extensions where the uniseriate portion of the ray extends for four or five ray cells. None of the rays observed in the tangential sections have storying (see Figures 20. and 21.).

Radial sections of *Xanthorhiza simplicissima* showed that all ray cells are upright, that is, the longest sides of the cells are oriented vertically (see Figure 22.). The mean height of a ray cell is 55.1 μm (n = 54; SE = 2.2),and the mean width is 22.4 μm (n = 54; SE = 0.9).

Fibers

The fibers which are found in the secondary xylem of *Xanthorhiza simplicissima* are libriform fibers (see Figure 23.). This fiber type characteristically has tapered ends, although forked ends can be found occasionally. In transverse sections the fibers are rounded or somewhat oval in cross-section, with small lumens visible. The fibers also have pit apertures which resemble slits, and have no apparent borders (simple pits). The mean length of the libriform fibers is 351.1 μm (n = 50; SE = 8.8), and the mean width (including the cell wall) is 15.9 μm (n = 50; SE = 0.5).

Libriform fibers are very abundant throughout the secondary xylem, and are distributed around both vessels and rays. Basically, the fibers form a matrix in which the vessels and rays are embedded within the xylem. Some of the fibers close to ray cells which contain the lumps of unidentified material appear to contain the same substance. Fiber cells farther away from the rays do not contain these rounded objects. No storying occurs in the libriform fibers (see Figure 21.).

The thesis data for *Xanthorhiza simplicissima* are summarized in Appendix A, Table 1.

Stem Anatomy and Xylem Characteristics of *Coreopsis gigantea*

Overview of the Stem Anatomy

Transverse sections of *Coreopsis gigantea* show a stem and xylem anatomy quite different from *Xanthorhiza simplicissima*. In general, ground tissue made up of parenchyma cells and containing vascular bundles of xylem and phloem tissue arranged in a ring pattern are the dominant feature of *Coreopsis gigantea* stems. This arrangement contrasts with the cylinder of xylem consisting of vessels and distinct xylary rays embedded in a matrix of fibers found in *Xanthorhiza simplicissima*. In addition, there are some differences between the stem and xylem anatomy of young (smaller) stems versus that of older (larger) stems in *Coreopsis gigantea*. While observations based on young stems were recorded, the paedomorphic related observations and measurements of the study were based on larger stems, since older stems have more substantial amounts of secondary xylem present.

Parenchyma in Younger, Smaller Stems

In the study small stems with radii of 9.5, 11.0, or 11.5 mm were used to analyze the stem and xylem anatomy of young stems. In these stems parenchyma cells throughout the transverse sections are thin walled. In the center of the stem the parenchyma cells are rounded or slightly oval in outline. Frequently they have flattened sides where the parenchyma cells make contact with other adjacent, surrounding parenchyma cells. In these cases there may be one, two, or three sides which are slightly flattened. The general

trend for parenchyma cells in transverse sections is that they become smaller moving from the stem center into the area where the vascular bundles are found, and then become larger again towards the outside of the stem. Parenchyma cells found towards the outer part of the stem tend to be smaller than those located in the stem center (see Figures 26. and 27.).

As a result, there are large, rounded parenchyma cells in the center of the smaller stems. The parenchyma cells make a transition to much smaller parenchyma cells as they approach the earliest formed vessels of the vascular bundles. In transverse views, the shape of the parenchyma cells as they approach the first formed vessels varies. In some cases they retain their rounded shape even if they lie directly beside the vessels. In other instances, they remain rounded up to a certain point (within 10 parenchyma cells or less from the vessels), and then they become more oval shaped or even elongated. An inner ring of secretory canals appears in the same area of the parenchyma tissue close to the earliest formed vessels (see Figure 28.).

Each of the vascular bundles of the ring has xylem towards the stem center, and phloem towards the outside. In transverse view, the vessels have a rounded or oval outline, with flattened sides common in both single vessels as well as vessels in groups. Around the vessels of the vascular bundles, some of the smaller parenchyma cells may be shaped like elongated ovals, while others may not be drastically elongated, but only slightly oval shaped (see Figure 28.).

In transverse views the elongated oval shaped parenchyma cells are most common where the areas of parenchyma between the vascular bundles (the interfascicular regions,

also known as medullary rays, pith rays, or interfascicular parenchyma) are narrow. In very narrow interfascicular regions of the smaller stems of *Coropsis gigantea*, not only are parenchyma cells with elongated oval shapes seen. Elongated oval shaped parenchyma cells with somewhat flattened ends, tending towards a rectangular shape, may be seen also in the most recently formed tissue. These somewhat rectangular shaped parenchyma cells persist into the phloem as a continuation of the interfascicular region (see Figure 29.).

On the other hand, if the interfasicular regions are wide, the parenchyma cells within those areas may remain rounded in outline, rather than being oval or shaped like an elongated oval (see Figure 30.).

Just outside the most recently formed vessels formed in each bundle lie strands of dark staining phloem embedded in parenchyma cells. A second ring of secretory canals lies outside the outer boundary of the phloem in the parenchyma, and the parenchyma cells located here make the transition back to more rounded outlines (see Figure 31.). That transition is reversed eventually, and the parenchyma cells become more oval shaped as they approach a third ring of secretory canals contained in the outer cortex just inside the periderm. These outer parenchyma cells are vertically oriented to parallel the outside surface of the stem.

Vessels in Younger, Smaller Stems

The vessels of the vascular bundles embedded in the parenchyma of the smaller stems are typically found in short wedge shaped or roughly rectangular groups, as seen in transverse sections. As indicated previously, some of the vascular bundles may lie close

to each other, with narrow interfasicular regions. Other interfasicular regions in the same stem section may be much wider. Within the vascular bundles of the smaller stems, usually the vessels are arranged in multiple, discontinuous vessel strands that radiate outwards. Sections of the strands may also exhibit tangential or diagonal patterns. Beyond this, however, in many cases it is difficult to determine a distinct pattern of distribution of vessels within the vascular bundles in the younger stems. For example, recognizable gaps in the vessel strands may or may not exist, and obvious differences in trends of the diameter size of the vessels may be hard to discern. But at least in some cases there may be gaps in the vessel strands which lie parallel to corresponding gaps in adjacent vessel bundles, which signal the start or stop of vascular growth. There may also be a trend of larger diameter vessels making a transition to smaller diameter vessel-but this is not always seen in the smaller, younger stem cross-sections (see Figures 31. and 32.).

Parenchyma in Older, Larger Stems

Large stems with a radius of either 26.0 mm or 27.5 mm were used to study the stem and xylem anatomy of older stems of *Coreopsis gigantea*, and were used as the basis for the measurements, as well as the observations and data pertaining to paedomorphosis. Many of the observations made about the transverse sections of younger stems hold true for the older stems, but there are some differences. In transverse sections of older stems, if there are wide interfascicular regions, there is the tendency for parenchyma cells located in the middle of the interfasicular region to be noticeably larger than those lying close to the vessels, as well as those among the vessels. Regardless of the

width of the interfascicular regions, the parenchyma cells next to vessels tend to be smaller in size, and the parenchyma cells tend to change from a rounded shape at the stem center to an elongated oval shape, and then to a somewhat rectangular shape at the youngest vessels. In contrast to the younger stems, not only do the parenchyma cells tend to be smaller among the more recently formed vessels, but they tend to be somewhat rectangular shaped two or three cells deep adjacent to the vessels, and among the vessels. In the area of the youngest vessels, many of the parenchyma cells in the interfascicular regions in transverse view may be elongated ovals with flattened ends, or rectangular shaped, to a greater extent than found in younger stems (see Figures 33. and 34.).

Vessels in Older, Larger Stems

There are also differences in the larger stems in the vessel portion of the vascular bundles. Transverse sections from older stems show vessels in larger stems clearly distributed in a semi-ring porous pattern. A single strand, or sometimes double strands (or more), typically extends radially from a cluster of vessels. The strand usually runs parallel to the interfasicular regions adjacent to the vessels (see Figures 35. and 36.). In the more mature stems, the interfasicular regions that lie between each vessel strand tend to be more consistently narrow when compared to younger stems, although there are exceptions (see Figure 37.).

In the older stems, the vessel clusters, which consist of larger diameter vessels (sometimes only marginally larger diameter vessels), have a tangential or sometimes a diagonal orientation in transverse sections. The clusters are not always well defined, and may consist of only one or two large diameter vessels at times. These conglomerations of

larger diameter vessels represent the earlywood (i.e. springwood).The vessel strand that extends from the tangentially oriented cluster represents the latewood (i.e. summerwood). The vessel strand is discontinuous, and consists of single vessels which do not make contact with nearby vessels, as well as groups of two or three vessels in contact with each other. Mean vessel group size is 1.7 vessels per group (n = 26; SE = 0.1), although the range can run up to 11 vessels in a group.

Normally there is a noticeable space where the vessel strands end in the parenchyma or a few fibers. After the space usually there is another tangential cluster of larger diameter vessels with another discontinuous vessel strand extending from it (see Figures 35. and 37.). Thus, in older stems the vessels exhibit a repeating pattern of tangential vessel cluster, radiating strand of vessels, and then a substantial break in the vessels. A vascular cambial zone can also be seen in the transverse sections as a darker staining, continuous ring where the vessel strands begin in the vascular bundles (see Figures 34. and 36.).

The vessels tend to show storying in tangential views (see Figures 38. and 39.). The vessel elements which compose the vessels have a mean length of 180.2 μm (n = 50; SE = 5.9), and a mean width of 51.6 μm (n = 50; SE = 1.8), as determined in maceration preparations. They frequently widen in circumference at their ends, and occasionally have slight caudate tips (see Figure 40.). Most often they have fairly transverse or perhaps slightly oblique (slanted or angled) end walls. A few of the vessel elements have helical lateral wall deposition patterns (see Figure 41.), but the majority are helical transitional to scalariform lateral wall deposition patterns. The tendency is towards a

scalariform lateral wall deposition pattern. The end walls have simple perforation plates. No lateral wall pits are visible on the vessel elements (see Figure 42.).

Vessel element lengths were measured across the width of the xylem, moving from xylem formed at the edge of the pith and moving towards the vascular cambium. Graphs of three sets of data points using either a Locally Weighted Scatterplot Smoother line or a linear regression line were prepared. The first graph, which uses vessel element lengths from younger stems, shows a decrease in vessel element length, with more recently formed vessel elements having shorter lengths than the older vessel elements. The resulting curve has an overall negative slope (see Figure 51.). When a linear regression line is fitted to the same data points, a negatively sloped line results also (p = 0.0 and r^2 = 10.1%) (see Figure 52.).

Plotting a second set of data points, taken from a stem with a 29.0 mm radius, also produced a negatively sloped curve (see Figure 53.). A linear regression line fitted to this data shows a negative slope (p = 0.0 and r^2 = 17.5%) (see Figure 54). A third set of data points, taken from a stem with 29.9 mm radius (but from different slide sections), led to a nearly flat curve when plotted (see Figure 55.). A linear regression line fitted to the third data set shows a nearly flat curve also (p = 0.67 and r^2 = 0.1%) (see Figure 56.).

Interfasicular Regions in Older, Larger Stems

Just like the young stems of *Coreopsis gigantea*, older stems also have interfasicular regions, which lie between the vascular bundles, rather than xylary rays. In radial sections of older stems where the vascular bundles closest to the stem center (i.e. the first formed xylem) can be observed, the parenchyma cells follow the same general

trends as seen in transverse views. The stem center has larger, rounded parenchyma cells. Around the earliest formed vessels, which tend to be single (or only two or three) isolated vessels (sometimes having helical lateral wall deposition patterns), the parenchyma cells are oval shaped or sometimes a little elongated, and tend to be oriented vertically (i.e. have an upright orientation). As vessels increase in number, there are still rounded parenchyma cells, but vertically oriented ones increase in number and become more common immediately adjacent beside vessels (see Figure 43.).

Parenchyma found in tangential sections of older stems tends to exhibit the same general tendencies as parenchyma in younger stems, but rounded parenchyma cells are much less common. Elongated oval shaped parenchyma cells in vertical orientation predominate. Parenchyma cells directly beside vessels and in very narrow areas of parenchyma cells between vessels are typically narrower than other parenchyma cells. There are also more of the elongated parenchyma cells with angled, slightly pointed ends, or parenchyma cells with flattened ends. Some of the parenchyma cells are coffin shaped (see Figures 44. and 45.).

None of the interfasicular regions were measured in tangential sections because they exceeded the height of the sections (5670.0 μm). However, these areas of parenchyma are multiseriate and nonstoried. Mean width of the most recently formed interfasicular regions is 134.5 μm (n = 20; SE = 17.5), or 5.6 cells (n = 20; SE = 0.50) (see Figures 38. and 46.).

Radial sections of older stems also revealed parenchyma cells in the most recently formed interfasicular regions that are rectangular in outline. Of these, most were upright

in orientation, with a few squarish cells observed. Only a few procumbent cells were seen. The mean height of the parenchyma cells found in the interfasicular regions is 113.2 μm (n = 50; SE = 3.7), while the mean width is 69.5 μm (n = 50; SE = 2.4) (see Figures 47., 48., and 49.).

Fibers in Older, Larger Stems

Libriform fibers are found in the xylem of *Coreopsis gigantea*. This type of xylary fiber has slit-like pit apertures with no apparent borders (simple pits). In transverse sections the fibers appear rounded or oval shaped in outline. Macerations show that most of the fibers are tapered (see Figure 50.), although examples of fibers with forked ends can be found occasionally. In maceration preparations, the libriform fibers have a mean length of 336.7 μm (n = 53; SE = 10.7), and a mean width of 27.1 μm (n = 53; SE = 1.1).

In general, fibers are not extremely common in *Coreopsis gigantea*. They are closely associated with the vessels, and normally are found next to single vessels or groups of vessels. If they are not immediately adjacent to a vessel, they are found parallel to the vessels, and separated by only a few parenchyma cells. Fibers are not found isolated in areas of parenchyma far from the vessels. Wherever they are located in the xylem, they take the form of either strands of single, attached fibers or strands that are 2-4 fibers wide. Many of the multi-strand fibers have a degree of storying (see Figure 44.).

Other than vessels, libriform fibers, and parenchyma cells of varying dimensions, no other cell types were seen in the xylem. As a result, there is no evidence for tracheids or fiber-tracheids in the xylem of *Coreopsis gigantea*.

Thesis data for *Coreopsis gigantea* are summarized in Appendix A, Table 2.

Stem Anatomy and Xylem Characteristics of *Mahonia bealei*

Overview of the Stem Anatomy

The stem anatomy of *Mahonia bealei* resembles that of *Xanthorhiza simplisissima* somewhat. Like *Xanthorhiza simplicissima*, transverse sections of *Mahonia bealei* show a prominent pith composed of parenchyma cells in the stem center, which is surrounded by a cylinder of xylem. Just beyond the vascular cambium are arches of compressed phloem tissue mixed with parenchyma cells. The tissue where the phloem is found protrudes as a convex area into the parenchyma cells that lie to the inside of the periderm. This area of parenchyma also contains irregularly shaped groups of fibers, each centered over the convex protrusion of phloem and other tissue (see Figure 57.).

In the main part of the pith of the stem, in transverse sections parenchyma cells tend to be fairly large and rounded in outline, although some have a flattened side or two where adjoining parenchyma cells abut them (see Figure 58.). In radial and tangential views, the parenchyma cells are aligned in vertical files of cells where the cells are somewhat rectangular or squarish, and are oriented vertically (upright). Often the cell end walls are angled, or the side walls may be curved or not exactly parallel, so that the files or columns of parenchyma cells appear “curvy”. The pith cells usually have a large amount of some unidentified translucent, lumpy material in their interior, and some may have rhomboid shaped crystals inside them. They also appear to have thicker cell walls than the parenchyma cells found in the other two plants of the study (see Figures 59. and 60.).

In transverse sections the parenchyma cells of the pith in *Mahonia bealei* gradually get smaller as they approach the beginning of the xylem (the oldest formed xylem). They also begin to assume a more oval, slightly elongated outline just outside the xylem. The first formed vessels consist of a wedge (a v-shaped cluster) of vessels within a matrix of fibers. The parenchyma cells extend past these wedges of vessels to form a sort of arm on each side of the initial vessel cluster. Within each arm, the parenchyma cells make a transition from oval, slightly elongated cells to somewhat rectangular outlines. Typically by the time a strand of vessels emerges from the initial wedge shaped cluster of vessels (or by the first break in the vessel strand) the parenchyma cells have assumed the rectangular outline of xylary ray cells (see Figure 61.).

The xylary rays run the width of the xylem, and the rays continue into the phloem just beyond the vascular cambium before making a transition to rounded or oval parenchyma cells. The parenchyma cells found immediately around the series of arches of phloem (which are found lying between the phloem rays) are the smallest of all the parenchyma cells in the transverse sections of stem. These make a transition to larger, rounded or oval shaped parenchyma cells. These are oriented parallel to the surface of the stem, and are found between the phloem and periderm.

Vessels

Vessels are a prominent feature of the secondary xylem in *Mahonia bealei*. In transverse sections, they are rounded or oval in cross-section, with flattened sides common. The vessels are embedded in a matrix of fibers. They typically are distributed in a pattern where a discontinuous vessel strand emerges from a wedge or v-shaped cluster

of vessels formed just outside the pith. This strand consists of both large and small diameter vessels, which are found in groups of vessels in contact with each other, as well as single vessels. Clusters containing large numbers of multiple touching vessels are not uncommon (for example, vessel groups of 24, 33, and 63 were observed). However, single vessels and groups of two or three are found also. The mean number of vessels per group is 6.7 vessels (n = 25: SE = 0.3) (see Figure 62.).

The strand itself is broken into segments, which are separated by areas of the fiber matrix. There are normally larger diameter vessels forming the strand just after one of the breaks. Vessels later in the strand may only be marginally smaller in diameter. Since there are differences in vessel diameter, the vessel distribution can be called semi-ring porous (see Figure 63). No obvious growth rings marked by differences in staining or cell density have been observed. It is interesting that sometimes the breaks in the strands are roughly parallel between the vessel strands in a given section, as well as the patterns of parts of the vessel strands. However, this is inconsistent (see Figures 62. and 63.).

The discontinuous vessel strand usually zig-zags back and forth between two xylary rays as it extends towards the vascular cambium and phloem. Many of the sections of the strand lie in a diagonal orientation, but it can also extend radially, or have tangentially oriented clusters present. Contact with one of the rays is fairly common, with only one or two vessels in contact with a ray, or many vessels in contact. At other times the vessels may miss contact with a ray by only a fiber cell or two (see Figures 62. and 63.).

Vessel elements in macerations show a helical lateral wall pattern. The lateral walls have helical grooves, which lie between two slightly raised ridges of secondary wall deposition material. By adjusting the focus, lateral wall pitting can be seen on most vessel elements. The pitting is in the form of narrow oval pits (frequently slit-like) in an alternating pattern, and tends to follow the groove of the lateral wall. Light microscopy indicates that the pits are nonbordered (simple). However, this should be confirmed by other methods. The vessel elements also have simple perforation plates. The end walls tend to be at least moderately oblique (i.e. slanted or angled). Often one or both of the vessel element ends has a caudate tip. Sometimes the caudate tips are only slightly extended, but a few may be very extended. The mean length for vessel elements in *Mahonia bealei* is 257.5 μm (n = 50; SE = 9.2), and the mean width is 24.7 μm (n = 250; SE = 1.1) (see Figures 64., 65., and 66.).

Some vessel elements in tangential sections show a degree of storying. However, storying is not consistent, and many vessel elements do not exhibit storying (see Figure 67.).

A graph of vessel element lengths with a Locally Weighted Scatterplot Smoother line fitted to the data points shows vessel element length remains roughly the same across the xylem, with more recently formed vessel elements having similar lengths to those of older vessel elements. The resulting curve has a nearly zero slope (see Figure 76.). When a linear regression line is fitted to the same data points, a negatively sloped line results also ($p = 0.832$ and $r^2 = 0.0\%$) (see Figure 77.).

Tracheids appear to be present in *Mahonia bealei*, and are most commonly associated with smaller vessel elements. Thus, they are vasicentric tracheids. They have the same type of lateral wall deposition pattern and lateral wall pitting, but are long and narrow in diameter. Being tracheids, they do not have openings in their end walls, but instead show pointed ends (see Figure 68.). No measurements were made of the vasicentric tracheids.

Rays

Rays are another prominent feature of the secondary xylem in *Mahonia bealei*. In general, the xylary rays are multiseriate. In transverse sections the rays tend to start out a couple of cells wide, but a few start with a width of three or four cells, before widening. They run the width of the xylem, before ending at the vascular cambial zone. In tangential sections, most xylary rays are multiseriate, but there are examples of occasional uniseriate rays. Almost all the rays in the tangential sections extended beyond the limits of the margins of the sections, so their true height could not be determined. Few of the rays seen in tangential sections exhibit the convex shape commonly seen in the literature for rays, and their width is relatively unchanged along the height of the rays. Mean width of the xylary rays is 63.8 μm (n = 27; SE = 2.9), or 3.7 ray cells (n = 27; SE =0.126) (see Figure 69.).

Although tangential sections reveal that most of the rays are multiseriate, many of the multiseriate rays have uniseriate portions. These rays alternate between multiseriate width (typically two or three cells wide) and uniseriate width, for varying portions of the ray, and in no particular pattern. Often very short rays contained completely within the

section are uniseriate for their entire height. In the instances where the end of a ray could be seen in the section, the end of the ray may be uniseriate in the form of a single ray cell, or in the form of an extended uniseriate portion (up to five cells or longer) (see Figure 70.).

Rays also contain a mix of ray cell shape in tangential sections. Sections of a ray may contain either rounded ray cells, oval shaped ray cells, or extended oval shaped ray cells. A mix of these shapes may be seen in either the multiseriate portion of the ray, or the uniseriate portion. Often the oval shaped or extended oval shaped ray cells are found on the margins of the ray in an upright (vertical orientation), or in uniseriate portions of a ray. Also, there are fluctuations in ray cell size (volume), without any sort of pattern in the changes. None of the rays are storied (see Figures 66., 69., and 71.).

Rays in radial sections are a mix of both procumbent and upright cells, with no clear-cut domination by either type. Usually a row of ray cells remains consistent in cell type, but exceptions exist. In some instances several rows of one cell type will alternate with several rows of another type, but there does not seem to be a discernable pattern (see Figures 72. and 73.). Procumbent ray cells have a mean height of 25.5 μm (n = 30; SE = 1.0), and a mean width of 46.1 μm (n = 30; SE = 2.1). Upright ray cells have a mean height of 34.0 μm (n = 20; SE = 1.4), and a mean width of 31.4 μm (n = 20; 0.8). The mean height for all ray cells measured is 28.9 μm (n = 50; SE = 1.0), and the mean width is 40.3 μm (n = 50; SE = 1.7).

Fibers

The secondary xylem of *Mahonia bealei* contains many fibers, which surround the vessels and rays, and serve as a matrix for the other cell types. The fibers have simple pits on their lateral walls, and are considered libriform fibers. In transverse sections these appear as rounded or oval cross-sections. In macerations the fibers commonly are elongated with pointed ends (see Figure 74.). Mean length of the libriform fibers is 387.1 μm (n = 50: SE = 14.5), and mean width is 15.5 μm (n = 50; SE = 0.5). Some storying occurs in the libriform fibers of *Mahonia bealei*, but this is seen only in some instances where two, three, or four fibers adjacent to each other show a storying pattern. Typically storying in these situations does not persist into the greater surrounding area of fibers (see Figure 75.).

Thesis data for *Mahonia bealei* are summarized in Appendix A., Table 3.

CHAPTER V

DISCUSSION

Paedomorphosis in the Secondary Xylem

Carlquist (1962) used Bailey's refugium theory (1944) in developing his theory of paedomorphosis in secondary xylem. The refugium theory states that there is an evolutionary lag in development in the tracheary elements of the primary xylem, compared to those in the secondary xylem. According to the theory, vessels originated in the secondary xylem first, by evolving from the tracheids. Over time tracheary elements continued to evolve in the secondary xylem, becoming ever more specialized. Increasing specialization was evident through a directional series of structural changes in the tracheary elements. These changes included changes in lateral wall deposition patterns, modifications to vessel end walls, and increasingly shorter lengths in vessel elements. As a result of the lag in development in the primary xylem, the primary xylem tends to retain the earlier evolved characteristics. In contrast the secondary xylem contains tracheary elements with more specialized features. Thus, the primary xylem serves as a refugium (i.e. a refuge or haven) for less specialized (more primitive) vessel characteristics (Bailey 1944).

As is evident from the preceeding description, Bailey drew on the theory of the major trends of xylem evolution, which became known as the Baileyan trends. The trends

were formulated by plant anatomists over the first half of the twentieth century. They were meant to indicate the general direction of evolution in xylem characteristics. For the most part, the trends were thought to be irreversible. However, within specific plants one trend could be more or less advanced in relation to other trends (Bailey 1944).

One major trend of xylem evolution dealt with vessels. Early vessels evolved from tracheids. As vessels, they advanced from the more primitive annular and helical lateral wall deposition patterns to a scalariform pattern. Finally they reached the most advanced pattern, the pitted lateral wall deposition pattern. These changes were paralleled by a series of changes in the vessel end walls. More primitive (less evolved) vessels had bars across the opening of the end wall (scalariform perforation plates). More advanced vessels evolved with fewer bars, until eventually no bars were present (simple perforation plates). Vessels also evolved from possessing more primitive lateral wall pitting patterns. Over time the patterns became more advanced, moving from opposite pitting to alternate pitting. The vessel elements also became shorter over evolutionary time, and developed more transverse (less angled) end walls which did not overlap with the end walls of neighboring vessel elements (Frost 1930 a., 1930 b., 1931; Bailey 1944).

Trends in the evolution of the distribution of vessels were also investigated. Diffuse porous woods, where the vessel diameters remain relatively the same, were less advanced. The ring porous condition, found in woody angiosperms in northern temporate areas, contained clearly delineated areas of larger diameter vessels (earlywood) and areas of smaller diameter vessels (latewood). Ring porosity was more specialized or advanced (Gilbert 1940).

Another major trend in xylem evolution involved the changes in fibers. Fibers, like vessels, evolved from tracheids, but evolved towards a specialized support function. The less advanced form was the fiber tracheid, which has bordered lateral wall pitting like the tracheid. The more advanced form was the libriform fiber, which possesses simple pits (nonbordered pits) (Esau 1977).

Both wood and ray parenchyma were examined to determine trends. In the case of wood parenchyma, it was decided that the absence of wood parenchyma was the most primitive condition. Diffuse parenchyma (single cells scattered throughout the xylary fibers) was more advanced. Metratracheal parenchyma (wood parenchyma in concentric rings independent of vessels) was even more advanced. Vasicentric abundant parenchyma was considered the most advanced condition (Kribs 1937).

In ray parenchyma, heterogeneous rays possessing both upright and procumbent ray cells were thought to be primitive. More advanced rays had increasing levels of homogeneity. Homogeneous rays, consisting of only ray cells with procumbent orientation, were found to be the most advanced (Kribs 1937).

All the major trends just described were discovered by researchers using a couple of simple methods. The first method as to use the fossil record to determine how specialized a feature might be. For example if tracheids appeared in the fossil record before vessels, then the tracheids were thought to be ancestral (less specialized) than the vessels (Frost 1930 a., 1930 b., 1931; Bailey 1944).

The second method was the use of statistical correlation. A characteristic or cell type recognized as primitive due to its place in the fossil record could be compared to

other characteristics. If a given characteristic had a high correlation with the characteristic already recognized as primitive, then it too was primitive. For example, early researchers recognized that tracheids appeared in the fossil record before vessels. Thus they were considered primitive. In addition it seemed reasonable that vessels were derived from tracheids. They also knew (based on computed averages) that tracheids are longer than vessel elements. Therefore, any characteristics associated (correlated) with longer vessel element lengths would be primitive. On the other hand, a characteristic associated with shorter vessel elements would be more advanced (Frost 1930 a., b., 1931; Bailey 1944).

Use of statistical correlations was extended beyond vessels to other xylem components, such as xylary rays and xylary parenchyma. Patterns of parenchyma distribution in the xylem as well as ray composition type were both correlated with vessel characteristics (Kribs 1935, 937; Gilbert 1940).

One problem with using statistical correlation methods in this way is that they may just be tracking relative changes in the proportions of different taxonomic groups in the sampled fossil record at various times. For these methods to be valid, they should be conducted within a phylogenetic framework (Remington 2008).

Today the major trends of xylem evolution are still believed to be true, generally speaking. However, some of the assumptions underlying the trends limit their usefulness and application in plant anatomy. One basic assumption was that the trends follow a unidirectional, linear path (i.e. that they are irreversible). Another assumption was that water conductivity was the major driving force behind the evolution of the vessels. One

problem with these assumptions is that they tend to oversimplify a dynamic process. Another problem is that the assumptions do not explain why the trends occur very well. Since the major trends of xylem evolution serve as an underpinning to the theory of paedomorphosis in secondary xylem, it is good to examine any possible weaknesses in the assumptions behind the trends. More than anything else, Carlquist used the major trends as a point of reference, and his theory actually serves as a partial refutation to them.

Irreversibility of the major trends of xylem evolution can be questioned on the basis of several factors. First, there are several families with both simple and scalariform vessel perforation plates in different species. There are also families with both fiber-tracheids and libriform fibers. It can be argued that these illustrate the trends of xylem evolution. However, it is also possible to interpret the presence of the mix of primitive and advanced features in these families as a reversal of the major trends of xylem evolution. In other words, in these families some characteristics of the secondary xylem have reversed from a specialized or advanced condition to a less advanced condition (Bass and Wheeler 1996).

Another consideration is that there have instances where using parsimony as a guiding principle in constructing phylogenetic trees has led to the use of what might be considered a primitive character as a derived character. An example of this can be seen in the work done in reconstructing the phylogeny of angiosperms. In some studies (Donoghue and Doyle 1989; Loconte and Stevenson 1991) it was found to be more

parsimonious to consider lack of vessels as a derived character. Thus, in these phylogenetic studies there appear to be reversals of the major trends.

In addition, ecological wood anatomy has shown that certain environmental factors like seasonality, water availability, and temperature have strong correlations with wood structure (Endress et al. 2000). Other factors can also affect wood structure. In experiments with cacti such as *Cereus Peruvians* and *Cereus tetragonus* reduction of vessel density and vessel diameters was triggered by reducing the availability of nitrogen and phosphorous in the soil (Arnold and Mauseth 1999). While these may be only short-term responses, it seems reasonable that long-term selection pressure could reverse at least some of the major evolutionary trends.

Earlier researchers concentrated on measurements of width and length in their work on xylem evolution. The implicit idea behind this was that conductivity was the main driving force behind tracheary element evolution. However, we realize that while this is a very important factor, it is an oversimplification to consider it as the only determinant.

More recent ecological and functional studies have provided additional insights into xylem evolution. For example, in tracheary elements, there are competing trade-offs that shape how vessels evolve. There are inherent trade-offs between conductivity and safety. Wider vessels may offer greater conductivity, but they increase the risk of cavitation, the formation of bubbles in the water column from freeze-thaw cycles or drought (Hacke and Sperry 2001). Larger lateral wall pits enhance conductivity also, but they weaken the vessel wall [some researchers say lateral wall pits are more important

than lumen diameter in safety issues (Sperry 2003)]. The increased porosity of the pit membranes also offer increased conductivity, but this increases the risk of air seeding which causes cavitation (Sperry 2003). Thicker vessel walls offer greater safety against collapse caused by negative pressure. However, there is a greater cost to the plant in terms of energy since there is a greater investment in wall material (Sperry 2003).

Another driving force behind the evolution of tracheary elements is transpiration rate. Most of the water taken in by plants is lost due to the gas exchange that occurs during photosynthesis. Transpiration rate can be affected by several factors including size of leaves, the number of stomata, water availability, and CO_2 concentration in the atmosphere (Sperry 2003). There is evidence that early plants did not need highly conductive tracheary elements because of the much higher CO_2 levels in the atmosphere of that time. There was a highly favorable exchange rate for CO_2 versus water, so the cost of transpiration was greatly reduced. This explains the tracheary element morphology seen in early fossil plants. Others argue that ancestral characters were adapted to the high water availability and the low evaporative demand in the tropical, rainy environments of the early plants (Feild et al. 2004).

While some of the assumptions underlying the Baileyan trends can be faulted, the trends are generally true, and offer some insight into xylem evolution. However, Bailey's refugium theory is much weaker, and it does not explain adequately why primary xylem differs from secondary xylem. Primary xylem does not have the anatomical characteristics it has because it serves as some sort of evolutionary time capsule, as suggested by Bailey. Instead, it has those characteristics because they offer some

advantageous selective value to the young plant in its environment. During the period when the primary xylem serves as the main conductive tissue, young plants frequently have lower transpiration demands. This may happen for a variety of reasons. In some cases, the plants may be shaded in the understory of other more mature plants. They also have smaller masses of leaves with corresponding fewer stomata. They are also smaller, so that the fibers and heavier vessel lateral wall deposition patterns seen in the secondary xylem are not needed for structural support (Mauseth 1988).

Recent research on the genetic and cellular mechanisms behind secondary growth provides insight into the linkage between the primary xylem and the secondary xylem. It has been found that many of the mechanisms underlying the growth of the apical meristem also are involved with secondary growth. Plants like *Arabidopsis* which normally have only primary growth can be induced to produce secondary growth in the laboratory [interestingly enough, *Arabidopsis* does not commonly have xylary rays in any secondary xylem produced (Nieminen et al. 2004), which is a paedomorphic wood characteristic]. One study has shown that the gene sequences in loblolly pine responsible for secondary xylem growth have homologs in *Arabidopsis*. If this is the case, then the primary xylem has structural differences from secondary xylem partly because of the different expression of the same gene families (not because of lagging evolution) (Nieminen et al. 2004; Groover 2005).

Mabberly's (1974) criticism of the theory of paedomorphosis in the secondary xylem does not seem to be supported by the thesis research findings. He criticized the graphs of the vessel element lengths of *Macropiper excelsum* and *Talinum guadalupense*

used as support by Carlquist (1962) for his paedomorphic wood theory. According to Mabberly, the types of curves that were produced were due to the plants being pachycauls. Since they are pachycauls, their piths widen higher in the stem. As a result, with wide piths (with the resulting larger perimeter of the vascular cambium), the vessel element lengths necessarily level off or drop because intrusive growth in the cambial initials no longer play an important role in increasing the number of cambial initials. In contrast, a plant with conventional woodiness such as *Eriobotrya japonica* has a smaller pith (with a smaller perimeter for the vascular cambium as a result), and a curve developed from its vessel element lengths would show increased lengths early in the curve as intrusive growth elongated the initials (and subsequent vessel elements). Later as its vascular cambium enlarges, vessel element lengths decrease just as they do in pachycauls. Thus, Mabberly argued that the graphs of vessel element lengths taken from pachycauls captured only the later part of the curve seen for conventional woody plants (Mabberly 1974).

However, the problem with this argument is that two of the three plants in the thesis research are not pachycauls (*Xanthorhiza simplicissima* and *Mahonia bealei*). Yet in all three cases, graphs of their vessel elements from across the xylem produced curves comparable to those produced in Carlquist's (1962) pioneering study (see Figures 7., 24., 25., 76., and 77.). Thus, the data from the thesis research seem to weaken Mabberly's argument.

Secondary Woodiness

The fact that woody plants can evolve from nonwoody ancestors readily, and in a variety of environments (islands, equatorial highlands, and other areas) shows that xylem evolution is very plastic. It is also an indication that woodiness is not an either-or situation, but rather that woodiness can evolve along a continuum. From the available evidence, it is more useful to think of woodiness in terms of degree.

This is evident from recent work with plants like tobacco and *Arabidopsis*. Both of these plants normally do not produce secondary xylem. However, under special conditions they can be induced to grow secondary xylem. For example, if *Arabidopsis* has its inflorescences removed, and is grown under shorter periods of light, it will produce rayless wood (Oh et al. 2003). Work with new model systems like *Arabidopsis* has also led to a better understanding of why secondary woodiness occurs.

Both the apical meristem and the vascular cambium are related anatomically. The procambium is derived from the apical meristem. In turn, the procambium produces the vascular cambium, which then generates the secondary xylem and phloem (Ye 2002; Groover 2005). The two meristems are also linked by hormones like auxin and cytokinin. For example, auxin is transported from the apical meristem to start the formation of procambial cells (Ye 2002). There are also a number of genes such as STM which are expressed in both the apical meristem and the vascular cambium (Groover 2005).

The anatomical evidence, together with our emerging understanding of the genetic mechanisms behind xylogenesis, provides new insights into secondary woodiness. The implication is that for plants like *Arabidopsis*, the genes for secondary

growth must already be present. What triggers the development of secondary xylem in these cases is change in the environmental conditions. This, in turn, alters gene expression to produce woodiness (Groover 2005).

An interesting question is whether *any* aspect of the genetics required for "normal" wood has been lost due to evolution away from herbaceousness towards woodiness (or vice versa) (Remington 2008). This could be explored by comparing the part of the genome responsible for xylogenesis from some plant like *Populus* (whose genome has been completely mapped) to the genetic counterpart in an herbaceous plant like *Arabidopsis*.

Xanthorhiza simplicissima

Vessels

In general the thesis data and observations for the xylem anatomy of *Xanthorhiza simplicissima* are in agreement with the information available in the literature. Both the thesis and the available study from the literature (Carlquist 1995 c.) agree that the vessels are distributed in a semi-ring porous pattern. In addition both found that vessels are restricted to the central part of the fasicular areas, with no contact with the rays (Carlquist 1995 c.)

The mean length of the vessel elements determined from the thesis data is 249.4 µm. This is much shorter than the mean length for vessel elements in dicotyledonous woods, 649.0 µm (Metcalfe and Chalk 1950). However, it is longer than the mean length of vessel elements in *Xanthorhiza simplicissima* given in the literature, 167.0 µm (Carlquist 1995 c.). There are also differences in mean width of the vessel elements (there

is no information available about the mean width for vessel elements in dicotyledonous woods). The mean width of the vessel elements calculated from the thesis data is 32.9 μm. This compares to the 20.0 μm mean width found in Carlquist's (1995 c.) study. Thus, both the mean length and width of the vessel elements measured using the thesis data seem substantially longer and wider than the measurements given in the literature for the species.

Another difference between the findings of the thesis and the available literature is the mean number of vessels per group. The mean number of vessels per group is determined by surveying a transverse section of the xylem, and simply counting the number of single vessels and the vessels making contact with each other. For example, a single, solitary vessel not making contact with any other vessels is counted as one. A group where three vessels are touching would be counted as three. Then the vessel counts are averaged. A mean of 2.5 vessels per group was calculated using the thesis data. In contrast, the literature lists a mean of 4.5 vessels per group for *Xanthorhiza simplicissima* (Carlquist 1995 c.). Therefore, the stem sections reviewed in compiling the thesis data had more solitary vessels and smaller numbers of vessels touching in vessel groups than the samples used in Carlquist's (1995 c.) study. The difference between the vessels per group mean from the thesis research and Carlquist's (1995 c.) research seems substantial, also.

While the differences in these vessel and vessel element characteristics are fairly substantial, unfortunately whether the differences are *statistically* significant cannot be confirmed through statistical analysis. One method of comparing two sample means

involves the calculation of a *t*-statistic. The t-statistic is then used to determine a *p*-value. If the *p*-value is low (normally .05 or less), then there is strong statistical evidence that the two means are significantly different. However, to calculate a *t*-statistic the sample standard deviation must be available. If necessary, the individual values for the perimeter of interest can be used to calculate the standard deviation. Once the standard deviation is calculated for both sample means, then the two standard deviations can be used to calculate the standard error for the difference between the two sample means. This is then used in the denominator of the *t*-statistic calculation. However, there is no standard deviation (or access to the individual values) available from the study taken from the literature. As a result, a *t*-statistic calculation cannot be done.

In any case, there seem to be real differences between the thesis and the other study for the means calculated for vessel element length and width, and number of vessels per group. This may simply be a consequence of the samples used. Given that the study from the literature used samples from only one site, and that only three sites were sampled for the thesis data, no true random sampling methods were used. As noted previously, this is a normal practice in plant anatomy studies. However, there may be enough variation within the species in adapting to local conditions that some anatomical characteristics will vary somewhat from site to site. While there is evidence that at least some characteristics of xylem anatomy like vessel diameter and density are affected by environmental conditions (Arnold and Mauseth 1999), it is not clear how plastic these characteristics are. In any case, it may be by sheer chance a site could be selected that would produce specimens with noticeably different measurements for some of the

anatomical features (a form of sampling error). This would be most likely when only a few sites are used to gather specimens (as in this case). Obviously, much more extensive sampling could reduce this risk.

Some researchers argue that the larger number of vessels per group and the narrower vessel element width found in the study from the literature would be advantageous in a drier environment (Mauseth 1988). Cavitation (introduction of air bubbles) is an increased danger in drier (or colder) areas. A larger number of vessels in a group offers greater protection against cavitation through redundancy-if a couple of vessels in a cluster suffer cavitation, the other vessels insure that conductivity continues. The narrower vessel element length may be less prone to cavitation than wider vessel elements. Thus, it may be that the plants sampled in the study from the literature came from a drier location than those included in the thesis study.

Response to the local environment possibly could explain why mean vessel element length is shorter in the study from the literature. In general, shorter vessel element length provides a lower level of strength to the stem, all other things being equal. It is not clear how local conditions would affect that characteristic. However, short vessel element lengths can also be an adaptation to more xeric environments (Carlquist 1985). Also, it is possible that if larger numbers of older stems were sampled for the other study, that would affect mean vessel element length. In *Xanthorhiza simplicissima* as the xylem grows the mean vessel element length decreases over time (see Figure 24.). Older plants would therefore contain a larger number of shorter vessel elements, and mean length would decrease as a consequence.

Rays

There is almost no information in the literature about ray cells, rays, and ray storying in *Xanthorhiza simplicissima*. As a result, ray cell measurements and other observations from the thesis cannot be compared to findings from the literature. However, the thesis findings that rays are multiseriate and are composed of mostly upright oriented ray cells are supported in the literature for the species (Carlquist 1995 c.).

Fibers

Fortunately, there is more information about xylary fibers available from the literature. The mean length of xylary fibers seems to be relatively close in both the thesis data and the data from the literature. The mean length of the fibers determined from thesis measurements is 351.1 μm. In the literature it is 342.0 μm (Carlquist 1995 c.). These mean fiber lengths for *Xanthorhiza simplicissima* are much shorter than the mean length for xylary fibers in dicotyledonous woods, 1317.5 μm (Metcalfe and Chalk 1950).

Other characteristics of the xylary fibers determined by the thesis observations agree with those given in the literature. Xylary fibers are the libriform type, and they are typically nonstoried. In addition they are found next to the rays and the vessels (Carlquist 1995 c.).

Comparisons between the thesis research and the data and observations contained in the literature for *Xanthorhiza simplicissima* are summarized in Appendix A., Table 4.

Paedomorphosis in the Secondary Xylem

Early in the thesis research, it was hypothesized that *Xanthorhiza simplicissima* would exhibit paedomorphosis in the secondary xylem. Based on the data and observations from the research, the hypothesis is supported. Trends in vessel element length, the presence of libriform fibers in the xylem, and the presence of upright ray cells provide evidence that paedomorphosis is present in the xylem (Carlquist 1962).

One manifestation of paedomorphosis in the secondary xylem is the decrease in vessel element length as the secondary xylem grows. As a result, graphing vessel element lengths produces a negatively sloped curve. This can be seen in the thesis results.When the vessel element lengths for *Xanthorhiza simplicissima* are graphed, the plot of the data points produces a negatively sloped curve (see Figure 24.).

A negatively sloped curve also results when a linear regression line is fitted to the data points of the vessel element lengths (see Figure 25.). However, it must be noted that there is only a weak linear association between vessel element length and the distance of the vessel elements from the start of the xylem. One indication of this is that the square of the correlation coefficient (r^2) is only 10.4%. This reveals that only 10.4% of the variation in vessel element length (y) is explained by the straight line relationship between vessel element length (y) and the distance of the vessel element from the start of the xylem (x). Furthermore, the sample correlation coefficient (r) of the linear regression is only 0.32. The sample correlation coefficient measures the degree of linear association between vessel element length and the distance of the vessel element from the start of the xylem. Since 0.32 is closer to 0.0 (no linear association) than it is to 1.0 (perfect positive

linear association), this indicates there is a fairly weak linear association. However, *r* captures only linear relationships (this is true by extension to r^2 and linear regression in general). There may be a strong relationship between the two variables (vessel element length and the distance of the vessel element from the start of the xylem), just not a linear relationship.

As discussed previously, decreasing vessel element lengths over the width of the xylem are a sign of paedomorphosis. This can be explained by a review of the vessel element growth trends in typical woody dicotyledons. In typical woody dicotyledons, the earliest formed vessel elements of the secondary xylem continue the trend of decreasing length seen in the primary xylem. At some early point in the secondary xylem, the vessel element length begins to increase. Eventually as the secondary xylem matures, vessel element length levels off. Much later it may actually decrease slightly. In contrast, in some plants with paedomorphic secondary xylem, the vessel elements of the secondary xylem continue the trend of decreasing length indefinitely. Since it retains a "juvenile" characteristic seen mostly in the primary xylem of "normal" woods, secondary xylem with this characteristic has at least some degree of paedomorphosis (Carlquist 1962, 1989, 2001; Lens et al. 2005 a., 2005 b.).

Another manifestation of paedomorphosis seen in the secondary xylem of *Xanthorhiza simplicissima* is the presence of libriform fibers. Libriform fibers (which have simple pits) are the only type of xylary fiber found in *Xanthorhiza simplicissima*. They are not considered paedomorphic in the sense of being a primitive feature (typically found in the primary xylem of typical woody dicotyledons) that is extended into the

secondary xylem. After all, libriform fibers are considered to be more advanced from an evolutionary viewpoint. The major trends of xylem evolution hold that fiber-tracheids are derived from tracheids. In turn libriform fibers evolved from fiber-tracheids. Instead, libriform fibers are an indicator of paedomorphosis because they are associated with paedomorphic woods that show other characteristics more commonly found in the primary xylem (such as decreasing vessel element length). Few paedomorphic woods have tracheids, and only rayless paedomorphic secondary xylem typically have fiber-tracheids (Carlquist 1962, 2001; Lens et al. 2005 a., 2005 b.).

Xanthorhiza simplicissima has another characteristic which indicates paedomorphosis, rays consisting of predominantly upright ray cells. Square ray cells are also considered the equivalent of upright ray cells by plant anatomists (Carlquist 2001). In the thesis data, all ray cells that were measured in radial sections had dimensions reflecting an upright orientation.

The reason upright ray cells are indicative of paedomorphosis in the secondary xylem can be explained by Bailey's refugium theory (Bailey 1944) and the general trends of xylem evolution. [Both of these ideas were incorporated into Carlquist's theory of paedomorphosis (Carlquist 1962).] According to Bailey's refugium theory, more specialized features evolved first in the secondary xylem. These features evolved later in the primary xylem (Bailey 1944). As a result both the pith and primary xylem tend to have less specialized rays that consist of many upright (or square) ray cells. Secondary xylem containing rays that have mostly upright ray cells are said to retain a "juvenile' characteristic most commonly found in the primary xylem. Thus when this feature is

found in the secondary xylem, a degree of paedomorphosis exists (Carlquist 1962, 1983, 1989, 2001, 2003; Lens et al. 2005 a., 2005 b.).

In Kribs' contribution to the ideas underlying the general trends of xylem evolution, he developed a classification system for rays. The system grouped rays in categories based on increasing specialization. According to Kribs, dicotyledonous woods containing rays followed a linear path of evolution. Xylem containing both multiseriate rays and uniseriate rays was the first to evolve. The multiseriate rays consisted of upright and procumbent ray cells, and the uniseriate rays consisted of mostly upright ray cells. This ray type was considered the least specialized (the most primitive). Other ray types evolved into ever increasingly specialized rays. The most specialized was a ray which had only uniseriate rays of procumbent ray cells. In the ray classification system, upright ray cells indicate a more primitive system than procumbent ray cells (Kribs 1935).

Secondary Woodiness

It was hypothesized that *Xanthorhiza simplicissima* would possess secondary woodiness. This would be supported by its place within the phylogenetic relationships of related plants. The relationships shown in the phylogenetic tree taken from the literature support the hypothesis (see Figure 78.).

As seen in Figure 78., the outgroup *Euptelea* (a deciduous tree) is basal to the rest of the tree, which consists of genera belonging to the order Ranunculales. Based on the literature, the ancestral condition for the order is woody (Kim et al. 2004). In addition, the ancestral state for the other eudicots of the study (which are not included in the

phylogenetic tree used in the thesis research) is also the woody habit (Kim et al. 2004). As a result, the ancestor to *Euptelea* was coded for the woody character state.

Within the tree there are three character state changes to a nonwoody habit (see Table 7. to see how the habit for woodiness was established for each genus). One of these represents the ancestor to a group of mostly herbaceous perennials. Within this group lie *Xanthorhiza simplicissima* and *Nandina domestica*. Both of these plants have woody characteristics (see Table 7.). Given their nested position within a group of nonwoody genera, their woodiness must be secondarily derived.

Environmental Factors and Anatomy

Several of the xylem characteristics of *Xanthorhiza simplicissima* can be considered adaptations to its environment. It is most frequently found in moist areas around streams in the understory of mature forests. Although it is found in the coastal plain and piedmont, it is most common in the Appalachians in a belt running from the deep southern United States into New England (Kartesz 2008). In this area, the climate produces clear cut growing seasons where spring and early summer bring higher temperatures and increased rainfall. Later in the year lower temperatures cause a halt in growth.

The semi-ring porous distribution of vessels, where the vessels of the springwood have larger diameters compared to later vessels, is a response to the seasonal fluctuation of rainfall and temperature. In addition, the relatively low number of vessels per group indicates that the added safety of redundant vessels in groups is not needed in the moist, shaded conditions. Given the conditions of heavy shade and moist soil where it is found,

it is likely that transpiration rates do not require high vessel conductivity. Another indication that transpiration may not be excessive is that the leaves are fairly small and offer a small area available for photosynthesis. Thus, the vessel mean width is much lower than those of the dicotyledons at large (Metcalfe and Chalk 1950).

Its paedomorphic features-decreasing vessel element lengths, libriform fibers, and the presence of rays with upright cells-seem to indicate an adaptation in providing intermediate mechanical strength. Although the libriform fibers and vessel lateral wall deposition patterns create a dense wood, the rays and short vessel elements tend to offset the increased strength provided by the fibers and vessel walls. One indication of this is that many of the plants seen in the field are somewhat procumbent, especially as they grow taller. This is perhaps an example of the hypothesis that paedomorphosis represents a relaxation of selection for mechanical strength (Carlquist 2001).

Coreopsis gigantea

Vessels

Data and observations about the secondary xylem of *Coreopsis gigantea* are not as plentiful in the literature as they are for *Xanthorhiza simplicissima*. Despite this, the available information from the literature is largely in agreement with the results of the thesis research.

According to the thesis research, vessels in *Coreopsis gigantea* are distributed in a semi-ring porous pattern. This finding is supported in the literature (Carlquist 1985). However there is no discussion of vessel storying in the literature. However, the thesis observations found vessels in *Coreopsis gigantea* tend to show storying.

There is no mention of restriction patterns for vessels in *Coreopsis gigantea* in the literature. This is reasonable since the patterns are found only in woody dicots where the xylem contains rays. Restriction pattern refers to the pattern of distribution of the vessels between the rays in transverse sections. In the case of *Coreopsis gigantea*, its xylem does not have true rays. True xylary rays normally lie within a matrix of fibers, which may also contain vessels, tracheids, and axial parenchyma cells. Instead, the vessels are embedded in a matrix of parenchyma cells, along with a few fiber cells. Interfascicular regions of parenchyma separate the vascular bundles containing the vessels. Obviously, since there are no xylary rays, there can be no restriction patterns for the vessels.

The mean length of the vessel elements calculated for the thesis data is 180.2 μm. Mean width is 51.6 μm. Both of these measurements are drastically different from the mean length and mean width given for vessel elements in dicotyledonous woods in the literature. Mean length for vessels in dicotyleonous woods is much longer, 649.0 μm. Mean width is much wider, 94.0 μm (Metcalfe and chalk 1950). Measurements given in the literature for the species itself are much closer to those calculated for the thesis data. Mean length for vessels from *Coreopsis gigantea* is 210.0 μm. Mean width for vessels from *Coreopsis gigantea* is 36.7 μm (Carlquist 1985). These measurements from the literature seem substantially different from those calculated from the thesis data. However, as in the case of *Xanthorhiza simplicissima*, whether these are statistically significant differences cannot be supported with absolute certainty. The information necessary for statistical analysis simply is not available from the literature.

Mean number of vessels per group is 1.7 vessels, according to the thesis data. In contrast the literature lists a mean number of 2.2 vessels per group. Again, this seems like a substantial difference, but this cannot be proven without additional information.

What accounts for the differences in vessel element mean length and width and number of vessels per group remains an open question. Just like *Xanthorhiza simplicissima* there was no true random sampling done in either the thesis research or the literature study. As a result, the risk of sampling error is greatly increased, and could play a role in accounting for the different measurements.

Similar to the situation with *Xanthorhiza simplicissima*, two data sets of vessel element lengths taken from the thesis research show a drop in length for *Coreopsis gigantea* as the distance from the beginning of the xylem increases. If more samples were taken from larger, older stems in the thesis research compared to the literature study, then a shorter mean length could result in the thesis study. However, no details on the age or size of the plants sampled in the literature study are available.

In general, plants adapt to more xeric conditions over time by evolving more numerous vessels per mm^2 and narrower and shorter vessel elements, among other features (Carlquist 1985). The narrower width of the vessel elements and higher number of vessels per group could be an adaptation to more xeric conditions by the plants sampled in the literature study. However, if this were the case, then the mean vessel element length should also be shorter, which is not the case. As a result, this conjecture is unlikely to explain the differences.

Another possibility is that one of the studies may have used cultivated plants, while the other used material from the wild. Cultivated material may have more mesomorphic characteristics than wild material. However there is research that shows any difference due to this are not very great (Bissing 1982). The inconsistent trends among the three measurements also do not support this idea.

Other observations from the thesis research provide additional details about the vessel elements in *Coreopsis gigantea*. All the vessel elements viewed have simple perforation plates. However, there is some variability in lateral wall deposition patterns, depending on the age of the vessels. Vessels with helical lateral wall deposition patterns are found in the earlier formed xylem. On later formed vessels (the majority of the vessels) a helical transitional to scalariform (tending towards scalariform) secondary wall deposition pattern is found. There is no lateral wall pitting in the sense of conventional pitting types (scalariform, transitional, opposite, and alternating). Instead, perhaps the best way to refer to its pitting pattern is to use the term pseudoscalariform pitting, as described by Carlquist (2001).

In the conventional lateral wall pitting types, scalariform pitting refers to a phenomenon seen in vessels that have flattened sides, or facets. These sides may have a series of short pits that fit the width of the flattened side. The pits run down the length of the flattened sides, much like the rungs of a ladder (Esau 1977). *Coreopsis gigantea* clearly does not have this pattern of lateral wall pitting. Instead, pseudoscalariform pitting may be a better categorical term. In this type of pitting, smaller pits act as wedges to split other longer pits, many of which extend around the circumference (Carlquist 2001).

Carlquist's study (1985) is mixed in its support of the vessel element characteristics just described from the thesis research. It agrees that simple perforation plates are predominant. However, it found no helical sculpturing (defined as secondary wall thickening or grooves connecting the pit apertures) present as a secondary wall deposition pattern (Carlquist 1985). Another earlier study by Carlquist contradicts this. It found that the vessel elements have scalariform or near scalariform lateral wall pitting (Carlquist 1974).

These differences can probably be ascribed to the inconsistent (and therefore, confusing) use of some anatomical terms related to vessel element secondary wall deposition patterns and vessel element secondary wall pitting type. In plant anatomy, typically secondary wall deposition patterns are categorized as annular, helical, scalariform, reticulate, and pitted (excluding transitional forms, which are common). In these forms, the area of the primary wall (which is on the outside of the vessel element) not covered by the patterned secondary wall is available for diffusion. Lateral wall pitting itself can be in a scalariform, opposite, or alternating pattern (and the pits can be bordered or nonbordered). It is independent of the lateral wall deposition pattern (with the exception of the pitted secondary wall deposition pattern) (Esau 1977; Mauseth 1988).

While the two terms (lateral wall pitting and lateral wall deposition patterns) describe two different things, they may exist in combination. For example, it is conceivable that a vessel element can have a helical secondary wall deposition pattern, as well as an alternating secondary wall pitting type. In this case the primary wall would be exposed by openings in the secondary wall (pit apertures) that lie in an alternating

pattern. Overlaying this would be additional secondary wall material in the form of a helical shaped, raised band.

Common usage of the term scalariform in the literature tends to conflate the terms scalariform secondary wall deposition pattern and scalariform secondary pitting type. Strictly speaking, these are two different concepts. Scalariform secondary wall deposition pattern refers to the raised deposits of secondary wall material. In contrast, scalariform secondary wall pitting refers to the pattern of the pits themselves. Often, however, these terms are used interchangeably, which can result in confusion. In the case of *Coreopsis gigantea*, the literature probably describes the same phenomenon in vessel element characteristics as the thesis research.

Interfasicular Regions

Although there are no true xylary rays or ray cells present in *Coreopsis gigantea*, the mean width of the interfasicular regions located in the most recently formed xylem was calculated from the thesis data. In addition, the parenchyma cells in these areas were measured and characterized for cell orientation. However, no information related to this is available from the literature.

Fibers

Xylary fibers were measured and described in the thesis research. Unfortunately there is no information given in the literature about fibers in *Coreposis gigantea*.

Comparisons between the thesis research and the data and observations contained in the literature for *Coreopsis gigantea* are summarized in Appendix A., Table 5.

Paedomorphosis in the Secondary Xylem

It was hypothesized that the secondary xylem of *Coreopsis gigantea* is paedomorphic. The data and observations from the thesis research support the hypothesis in several ways. The trends in vessel element length, the pattern of secondary lateral wall pitting, perforation plate type, the nature of the parenchyma cells found in the xylem, fiber type, and the absence of rays all provide evidence of paedomorphosis (Carlquist 1962).

There are two manifestations of paedomorphic wood that involve trends in vessel element length. The first manifestation is the decrease in vessel element length as the secondary xylem grows (Carlquist 1962, 1989, 2001; Lens et al. 2005 a., 2005 b.). Not only is this seen in the vessel elements in *Xanthorhiza simplicissima*, but it is seen in *Coreopsis gigantea* as well. When the vessel element lengths are graphed, the plot of the data points produces a negatively sloped curve. As seen in Figure 51, this is the case for the vessel elements from small young stems. The same kind of curve was produced from a graph of vessel element lengths from larger, older stems. Linear regression lines fitted to the same data points also show a negative slope (Figures 52. and 54.). However, just as in the case of *Xanthorhiza simplicissima*, the squares of the coefficient (r^2) for both linear regressions are low values (10.1% and 17.5% respectively). When these are converted to *r* values (0.317 and 0.418 respectively), it is obvious that there is not a strong linear association between vessel element length and the distance from the start of the xylem (for a strong positive correlation the values would have to approach 1.0). However, there

may be another kind of relationship between the two variables, just not a linear relationship.

The other way that vessel element length can indicate paedomorphosis in the secondary xylem is the maintenance of roughly the same vessel element length as the xylem grows. Frequently the vessel elements will decrease in length early in the secondary xylem. Later the vessel element lengths stabilize, and the same length is maintained throughout xylem grow. Because the vessel element length never increases as it does in the secondary xylem of typical woody dicotyledons, secondary xylem with this characteristic retains a "juvenile" feature (Carlquist 1962, 2001). This trend in vessel element length is seen in another data set from larger, older stems of *Coreopsis gigantea* (Figure 55.). A linear regression line fitted to the data points gives a zero sloped curve (Figure 56.). The linear regression of the data set also gave a low r^2 value (0.1%) and r value (0.03). The p-value is also insignificant (0.666).

It is unclear why data sets of measurements taken from stems of the same diameter would produce very different types of curves. However, given that both sets differ in the number of measurements taken at the intervals over the distance across the xylem, the line smoother routine (Lowess) was probably affected. As mentioned in the Methods section, the Locally Weighted Scatterplot Smoother uses the closest surrounding data points to perform a weighted line regression for each data point. With different concentration patterns of data points, different curves will be necessarily fitted.

Another possible explanation for the derivation of different curves is that this may reflect heterogeneity between different stems. By sampling many different stems of

different size classes, additional data could provide evidence for one of the explanations (Remington 2008). In any case, the vessel element length data in all three graphs indicate paedomorphosis.

Another characteristic common to paedomorphic secondary xylem is scalariform or pseudoscalariform lateral wall pitting. Regardless of the use or misuse of terminology discussed earlier, it is clear that the *Coreopsis gigantea* vessel elements have the pitting features described (and illustrated) as pseudoscalariform by Carlquist (2001).

According to Bailey's refugium theory (1944) the primary xylem tends to have vessels with less specialized lateral wall pitting, such as scalariform pitting. Since this characteristic is more commonly found in the primary xylem, when it is found in secondary xylem, it is considered "juvenile" or paedomorphic (Carlquist 1962, 1997, 2001).

The vessel elements in *Coreopsis gigantea* also have simple perforation plates. By itself, it is not an indicator of paedomorphosis. However, it is usually associated with the paedomorphic characteristic of pseudoscalariform or scalariform lateral wall pitting. Together these features compose a paedomorphic indicator (Carlquist 1962, 1997, 2001; Lens et al. 2005 a., 2005 b.).

Abundant parenchyma cells, which compose the only axial component (aside from vessels) in the xylem, are another indicator of paedomorphosis in the secondary xylem. In many typical woody dicotyledons, the primary xylem has abundant parenchyma. In contrast the secondary xylem consists of mostly fibers or tracheids, instead of parenchyma. If extremely abundant parenchyma in the secondary xylem is

found, then there has been a continuation of the structure of the primary xylem. When this condition is found, a degree of paedomorphosis is possible (Carlquist 1962, 2001; Lens et al. 2005 a., 2005 b.). According to the thesis research, parenchyma cells are extremely common in the secondary xylem of *Coreopsis gigantea*.

While xylary fibers are not common in *Coreopsis gigantea*, the fibers that exist can best be described as libriform fibers (which possess unbordered, or simple pits). Libriform fibers are frequently associated with paedomorphic wood (tracheids are relatively rare in secondary xylem possessing paedomorphosis). Thus, they serve as another indicator of paedomorphosis (Carlquist 1962, 2001; Lens et al. 2005 a., 2005 b.).

Raylessness in the secondary xylem is also considered a manifestation of paedomorphosis. Since rays never develop in the secondary xylem, a permanent condition of arrested development exists for the rays, and their absence is considered "juvenile" or paedomorphic (Carlquist 2001; Lens et al. 2005 a., 2005 b.). Observations from the thesis research reveal that no true xylary rays exist in *Coreopsis gigantea*. Instead, interfasicular regions of parenchyma separate the extended vascular bundles. As a result the raylessness of *Coreopsis gigantea* provides evidence of some paedomorphosis in its secondary xylem.

Secondary Woodiness

It was hypothesized that *Coreopsis gigantea* is secondarily woody. The hypothesis is supported by a phylogenetic tree taken from the literature. In the tree, the relationships between *Coreopsis* species found in the western United States, Mexico, and Central America are depicted (Figure 79) (Archibald et al. 2005). Using information

taken from the literature and other sources, each species on the tree was coded woody or nonwoody (see Table 8.). Character state changes were also recorded on the tree.

Deciding character state changes basal to the western clade containing *Coreopsis gigantea* (the top eight species in the tree) was somewhat problematic. For example, information from the literature concerning whether the ancestor to the outgroup *Dahlia coccinea* was woody or nonwoody is conflicting. One study constructed a phylogeny based on chloroplast DNA restriction site mapping. The phylogeny provides support for ancestral woodiness for Asteraceae. In this particular phylogenetic tree, the Tribe Barnadesiinae was found to be basal to the other tribes of Asteraceae. Since 97% of the species in Barnadesiinae are woody, the predominance of woodiness compared to the other tribes was considered evidence for ancestral woodiness (Jansen et al. 1991).

Another phylogenetic tree of *Coreopsis* species was based on ITS sequences. It used two outgroups basal to *Coreopsis*, *Fitchia speciosa* and *Dahlia coccinea*/*Dahlia macdougalli* (Kim et al. 1999). *Fitchia speciosa* is a tree (Kartesz 2008), and is basal to *Dahlia*. *Dahlia* is nonwoody (Vivar-Evans 2006; Harris 2008; Saylor 2008). A more recent study that used nuclear ribosomal DNA to construct a phylogeny of Heliantheae supports an ancestral perennial herbaceous state for *Coreopsis* (Baldwin et al. 2002).

Given the conflicting information, the ancestor to the most basal member of the tree used in the thesis research (*Dahlia coccinea*) was coded as nonwoody (total number of character state changes for the overall tree were the same regardless of coding for the ancestor). Other changes in character state then followed. In any case, the ancestor to the western clade in which *Coreopsis gigantea* resides must have been nonwoody (this

results in the most parsimonious number of steps) (see Figure 79). As a result, the phylogenetic relationships show that *Coreopsis gigantea* has secondary woodiness.

Environmental Factors and Anatomy

Coreopsis gigantea is found on the Channel Islands of southern California and along a narrow strip of the mainland. It usually grows on cliffs and sand dunes in this area, exposed to the some of the effects of the nearby ocean. In general this area has a Mediterranean climate year round. Temperatures are relatively stable, but there are seasonal differences in the weather (Hall 1993; Kartesz 2008).

Some of the xylem characteristics of *Coreopsis gigantea* seem to be an adaptation to the climate and habitat. While its vessels have a semi-ring porous distribution, it can be difficult to distinguish larger diameter vessels from smaller diameter vessels in transverse sections (although there are definite differences in diameter). In addition, there are no true growth rings marked by heavier staining. Instead, there are breaks in the vascular strands indicating seasonal breaks in growth. In addition, the low number of vessels per group indicates a possible adaptation to mesic conditions (although it is possible that the large amount of parachymatous tissue helps to store water). Its shallow root system (see Figure 4.), fern-like leaves, moderate vessel widths also indicate a lesser need for high vessel conductivity. All of this shows an adaptation to a mild climate without radical seasonality.

Of the three plants in the thesis, *Coreopsis gigantea* has the highest number of paedomorphic features. As mentioned in the case of *Xanthorhiza simplicissima*, paedomorphosis is hypothesized to represent a release from selection for mechanical

strength (Carlquist 2001). Several of the xylem characteristics in *Coreopsis gigantea* which are paedomorphic support this assertion. Although it may have several stems or branches, it is not a tall plant, and its leaves and stems do not offer much wind resistance. As a result, its need for mechanical strength is minimal. The abundant parenchyma in its stem offers sufficient turgor to support the stem. In addition, its rayless condition probably adds additional strength, along with the pseudoscalariform lateral wall pitting in the vessels. In conventional woods, raylessness may weaken the wood since ray parenchyma offers less strength than the fibers they displace. However, in *Coreopsis gigantea*, fibers are uncommon and raylessness may add strength since the parenchyma cells of the interfasicular areas offer additional areas of turgor for support. This offsets the paucity of libriform fibers and the weaker declining or stable vessel element lengths.

Mahonia bealei

Vessels

The results from the thesis research for *Mahonia bealei* generally parallel the findings from the literature. For example, both the thesis research and the literature show that vessels are distributed in a ring-porous pattern. Vessels are typically not restricted to the center of the fasicular area, but make frequent contact with the rays. Both sources also agree that there is some limited vessel storying (Carlquist 1995 a.).

Mean length of the vessel elements calculated from the thesis data is 257.5 μm for *Mahonia bealei*. This is much shorter than the mean length for dicotyledonous woods from the literature, 649.0 μm (Metcalfe and Chalk 1950). However, it is comparable to the mean length calculated for the species in the literature, 272.0 μm (Carlquist 1995 a.).

The mean width of the vessel elements calculated for the species using thesis data is 24.7 μm. This is much shorter than the mean width of vessel elements as a whole in dicotyledonous woods, 94.0 μm. In contrast, the mean width (lumen diameter only) for *Mahonia bealei* vessel elements in the literature is 13.0 μm (Carlquist 1995 a.). Mean vessels per group determined from the thesis data is 6.7 vessels. The literature gives a mean of 11.8 vessels per group (Carlquist 1995 a.).

As before, the differences between the thesis and the literature measurements seem relatively large (although the information for statistical testing of the means is not available from the study from the literature). The lower mean width of the vessel elements (even considering only the lumen was measured) and the higher number of vessels per group found in the literature study could indicate greater xeric adaptation. However, the greater mean length for the vessel element found in the literature is not consistent with this trend. Also, as discussed before, differences in water availability for cultivated plants versus wild plants may not lead necessarily to greatly increased mesomorphic characteristics (Bissing 1982). In addition there is no information from the literature study about where the sampled plants were grown. As discussed previously, it is possible that sampling error or use of stems of a different size or age could account for these differences. Human error in measuring the cells, of course, could also account for the differences in mean measurements.

Other vessel element characteristics are in closer agreement between the thesis research and Carlquist's study (1995 a.). Observations from the thesis research as well as from the literature study agree that simple perforation plates are common in *Mahonia*

bealei vessel elements. In addition, both a helical secondary lateral wall deposition pattern and alternate secondary wall pitting are seen in the vessel elements (Carlquist 1995 a.).

Rays

Unlike the thesis research, ray cell dimensions were not measured in the literature study. However, Carlquist's study (1995 a.) agrees with the thesis results that rays are made up of a mix of procumbent and upright ray cells. Both also found that rays are multiseriate and are nonstoried (Carlquist 1995 a.).

Rays were not measured during the research done for the thesis. This was not possible because the rays extended past the height of the sections. However, ray height was estimated in the literature study based on rays contained within the tangential sections. Ray height was estimated as 2400.0 µm (Carlquist 1995 a.). This estimate seems low. Maximum section height (which the rays exceeded) from thesis observations was 5670.0 µm.

Mean width of the *Mahonia bealei* rays measured during the thesis research is 63.8 µm, or 3.7 cells. This compares to a ray width of 4.4 cells measured in the literature study (Carlquist 1995 a.). The difference between the two means seems fairly large, but its underlying reason (like previous measurements) cannot be readily explained. The ray width was not measured in µm in the study from the literature.

Fibers

There was also a noticeable difference between the mean length of fibers measured in the literature study and the thesis research. Calculations from the thesis data

produced a mean of 387.1 μm for fiber length. This is much shorter than the mean length calculated in the other study, 541.0 μm (Carlquist 1995 a.). In addition, it is shorter than the mean length calculated for fibers in dicotyledonous woods, 1317.5 μm (Metcalfe and Chalk 1950). Mean fiber width from the thesis research is 15.5 μm. This measurement was not calculated in the other study.

Other fiber characteristics were in general agreement between the thesis research and the study from the literature. Both studies found that the xylary fibers are libriform fibers (have nonbordered pitting) (Carlquist 1995 a.). These fibers are plentiful, and serve as a matrix for the vessels in the xylem. In the thesis research, some limited fiber storying was found. This, however, does not persist beyond a few adjacent fibers typically. The study from the literature indicates that no fiber storying occurs (Carlquist 1995 a.). In this situation, it is likely that the difference in observations about storying in the fibers is due to differences in interpretation.

Comparisons between the thesis research and the data and observations contained in the literature for *Mahonia bealei* are summarized in Appendix A., Table 6.

Paedomorphosis in the Secondary Xylem

It was hypothesized *that Mahonia* bealei would have paedomorphic characteristics in its secondary xylem. The hypothesis was supported by the thesis data and observations. However, while *Mahonia bealei* has a degree of paedomorphosis in its secondary xylem, it has the fewest paedomorphic features of the three species studied in the thesis. As a result, it may be considered to have only a minimal degree of paedomorphosis.

The first indication of paedomorphosis is the presence of stable vessel element lengths in the secondary xylem. After an initial drop, its vessel element lengths maintain roughly the same length as the secondary xylem grows (see Figure 76.). Since the vessel elements never increase in length as they do in typical dicotyledonous woods, this characteristic is considered "juvenile" or paedomorphic.

When a linear regression line is fitted to the data points of the vessel element lengths, a zero slope line results. (Figure 77.). As a result, there is not a strong linear relationship between vessel element length and distance from the start of the pith (r^2=0.0%). The lack of a linear relationship between vessel element length and distance from the start of the xylem in *Mahonia bealei* makes sense because the vessel element lengths stabilize and do not change in length across the xylem.

The second indication of paedomorphosis is the occurrence of libriform fibers in the secondary xylem. Observations show that these are extremely abundant. They basically form a matrix for the vessels and rays of the secondary xylem. Although they are not paedomorphic in the sense of being a more primitive feature found in the primary xylem, they are considered paedomorphic when found in conjunction with other paedomorphic features.

Secondary Woodiness

It was also hypothesized that *Mahonia bealei* has secondary woodiness. This is supported by the phylogenetic tree which shows the relationships among genera of Berberidaceae (Figure 80.).

In the phylogenetic tree, *Nandina* (which is woody) is basal to the other genera. In the larger phylogenetic tree (which includes species, rather than just genera) that the reduced tree used in the thesis was taken from, there are two outgroups basal to *Nandina*. One of these is *Akebia quinata*, which is a woody vine. The other member is *Glaucidium palmatum*, a nonwoody plant. However, to provide consistency between the trees used in the thesis research, the ancestor to *Nandina* was coded as nonwoody, just as it was coded in Figure 78. (Kim et al. 2004). By doing so, no additional steps were introduced into the phylogeny.

Mahonia and *Berberis* (both woody) are nested within a group of nonwoody genera, with *Ranzania* (nonwoody) branching off below them (see Table 9. to see how the woody habit was established for each genus). It appears that based on where character state changed, their woodiness must be derived from a nonwoody ancestor. As a result, indications are that *Mahonia bealei* is secondarily woody.

Environmental Factors and Anatomy

The genus *Mahonia* is found in the Pacific northwest and in Asia. Although *Mahonia bealei* is used widely as a landscaping shrub throughout the United States, it originated in China. However, it is unclear from what region in China it came. The anatomy of its xylem offers some clues to the sort of habit and climate it is adapted to.

Mahonia bealei has a semi-ring porous pattern of vessel distribution. This, together with its distinctive growth rings, show that it is adapted to a climate with a clearly delineated growing season. The first part of the growth ring consists of noticeably larger diameter vessels, which means growth is initiated with higher temperature and

plentiful water. Later in the growth ring the vessel diameters grow noticeable smaller, which indicates less rainfall.

It also has a higher number of vessels per group than the other two plants of the study. This, together with the presence of vasicentric tracheids found in some of the macerations, and fairly narrow vessels, may mean it has some additional margin of safety in case of fluctuations in water supply. Additional vessels in a cluster enable cavitated vessels to be by-passed. In addition, narrow vessels and tracheids are supposed to resist cavitation (Hacke and Sperry 2001). It also has a helical lateral wall deposition pattern in its vessels, which may allow for some expansion of the vessel walls. This feature could conceivably prevent implosion of the vessels due to extreme negative pressure resulting from dry conditions.

It is interesting that it has the fewest paedomorphic characteristics of the three plants studied. Indeed it is perhaps only marginally paedomorphic. However, considering the hypothesis that paedomorphosis represents a relaxation of selection for mechanical strength, this makes sense. In many ways its morphology demands mechanical strength. Although it has limited branching, it can grow very tall. Experience with attempts to section its stems in the laboratory demonstrates the hardness and density of its wood. Thus, there is an inverted relationship between degree of paedomorphosis and mechanical strength in *Mahonia bealei*.

CHAPTER VI

CONCLUSION

The theory of paedomorphosis in the secondary xylem is an explanation for trends found in the xylem of certain plants. The plants often are either evolving towards greater woodiness, or in some cases may be evolving away from woodiness. They may be found on islands or equatorial mountain areas, but this is not always true. In general they have a suite of xylem characteristics, many of which do not adhere to the major trends of xylem evolution found in typical woody dicotyledons. The characteristics include the decreasing or stabilizing length of vessel elements as the secondary xylem ages. Others are the presence of scalariform (or pseudoscalariform) lateral wall pitting with simple perforation plates in the vessels and the absence of rays or delayed ray development. Rays which consist of upright ray cells, parenchyma cells that form a dominant axial component of the xylem, and libriform fibers are also considered indicative of paedomorphic wood.

Often paedomorphosis is associated with secondary woodiness. Secondary woodiness refers to woody plants whose ancestors are nonwoody. Secondary woodiness cannot be assumed to result from paedomorphosis, and must be determined based on phylogenetic relationships.

In the thesis research the secondary xylem of *Xanthorhiza simplicissima*, *Coreopsis gigantea*, and *Mahonia bealei* was examined for the characteristics of paedomorphic wood. All three were found to have a degree of paedomorphosis, with

Coreopsis gigantea having the largest number of paedomorphic characteristics. *Mahonia bealei* has only a minimal degree of paedomorphosis, while *Xanthorhiza simplicissima* is intermediate. It is believed that paedomorphosis represents a relaxation of selection for mechanical strength (Carlquist 2001). This appears to be true for the three plants, for their apparent mechanical strength is inversely correlated with the degree of paedomorphosis.

Given that there seems to be an inverse relationship between mechanical strength and the degree of paedomorphosis, it is possible that the paedomorphic wood characteristics may be consequences of the plants' growth habits, rather than deriving from secondary woodiness. On the other hand, perhaps the growth habits have been limited by the paedomorphic characteristics of the secondary xylem (Remington 2008). Future research could explore these speculations by examining the correlation between paedomorphic characteristics and growth habit within a phylogenetic context.

In addition, the three plants were placed in the context of their phylogenetic relationships to determine if they possessed secondary woodiness. In each case, the plant has nonwoody ancestors, and is secondarily woody.

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APPENDIX A: TABLES

Table 1. Thesis Data for *Xanthorhiza simplicissima*

Cell or Tissue Type	Sample Size (n)	Mean Length (or Height) (µm)	Standard Error (SE)
Vessel elements	50	249.4	9.4
Ray cells (upright)	54	55.1	2.2
Rays	26	(1)	(1)
Fibers	50	351.1	8.8

Cell or Tissue Type	Sample Size (n)	Mean Width[2] (µm)	Standard Error (SE)
Vessel elements	50	32.9	1.6
Ray cells (upright)	54	22.4	0.9
Rays	26	150.3 (9.6 cells)	12.4 0.8
Fibers	50	15.9	0.5

Table 1. (Continued) Thesis Data for *Xanthorhiza simplicissima*

Cell or Tissue Type	Sample Size (n)	Mean Number Per Group[3]	Standard Error (SE)
Vessel elements	26	2.5	0.1
Ray cells (upright)	54	(4)	(4)
Rays	26	(4)	(4)
Fibers	50	(4)	(4)

(1) Most rays extended past the ends of sections and could not be measured. Maximum height of the sections was 6000 µm.
[2]Width measured at widest point.
[3]Mean number per group refers to vessels only. Sample size (n) = 26 vessel strands.
(4) Not applicable to the cell or tissue type.

Table 2. Thesis Data for *Coreopsis gigantea*

Cell or Tissue Type	Sample Size (n)	Mean Length (or Height) (µm)	Standard Error (SE)
Vessel Elements	50	180.2	5.9
Interfasicular Parenchyma Cells[1] (all cell types)	50	113.2	3.7
Interfasicular Parenchyma Cells[1] (upright)	48	115.0	3.6
Interfasicular Parenchyma Cells[1] (procumbent)	2	70.9	2.0
Interfasicular Parenchyma Tissue[1]	20	(2)	(2)
Fibers	53	336.7	10.7

Table 2. (Continued) Thesis Data for *Coreopsis gigantea*

Cell or Tissue Type	Sample Size (n)	Mean Width[3] (µm)	Standard Error (SE)
Vessel elements	50	51.6	1.8
Interfasicular Parenchyma Cells[1] (all cell types)	50	69.5	2.4
Interfasicular Parenchyma Cells[1] (upright)	48	68.0	2.0
Interfasicular Parenchyma Cells[1] (procumbent)	2	107.4	34.5
Interfasicular Parenchyma Tissue[1]	20	134.5 (5.6 cells)	17.5 (0.50)
Fibers	53	27.1	1.1

Table 2. (Continued) Thesis Data for *Coreopsis gigantea*

Cell or Tissue Type	Sample Size (n)	Mean Number Per Group[4]	Standard Error (SE)
Vessel Elements	26	1.7	0.1
Interfasicular Parenchyma Cells[1] (all cell types)	50	(5)	(5)
Interfasicular Parenchyma Cells[1] (upright)	48	(5)	(5)
Interfasicular Parenchyma Cells[1] (procumbent)	2	(5)	(5)
Interfasicular Parenchyma Tissue[1]	20	(5)	(5)

Table 2. (Continued) Thesis Data for *Coreopsis gigantea*

Cell or Tissue Type	Sample Size (n)	Mean Number Per Group[4]	Standard Error (SE)
Fibers	53	(5)	(5)

[1]No true xylary rays or ray cells were observed in the *Coreopsis gigantea* specimens. Instead, interfasicular regions are present. Measurements were taken from parenchyma cells from interfasicular regions beside new vessels in older stems.
(2) Most interfasicular regions extended past the ends of the sections, and could not be measured. Maximum height of the sections was 8000 µm.
[3]Width measured at widest point.
[4]Mean number per group refers to vessel elements only. Sample size (n) = 26 vessel strands.
(5) Not applicable to the cell or tissue type.

Table 3. Thesis Data for *Mahonia bealei*

Cell or Tissue Type	Sample Size (n)	Mean Length (or Height) (µm)	Standard Error (SE)
Vessel elements	50	257.5	9.2
Ray cells (all)	50	28.9	1.0
Ray cells (upright)	20	34.0	1.4
Ray cells (procumbent)	30	25.5	1.0
Rays	(1)	(1)	(1)
Fibers	50	387.1	14.5

Table 3. (Continued) Thesis Data for *Mahonia bealei*

Cell or Tissue Type	Sample Size (n)	Mean Width[2] (µm)	Standard Error (SE)
Vessel elements	50	24.7	1.1
Ray cells (all)	50	40.3	1.7
Ray cells (upright)	20	31.4	0.8
Ray cells (procumbent)	30	46.1	2.1
Rays	27	63.8 (3.7 cells)	2.9 (0.126)
Fibers	50	15.5	0.5

Table 3. (Continued) Thesis Data for *Mahonia bealei*

Cell or Tissue Type	Sample Size (n)	Mean Number Per Group[3]	Standard Error (SE)
Vessel elements	25	6.7	0.3
Ray cells (all)	(4)	(4)	(4)
Ray cells (upright)	(4)	(4)	(4)
Ray cells (procumbent)	(4)	(4)	(4)
Rays[4]	(4)	(4)	(4)
Fibers	(4)	(4)	(4)

(1) Most rays extended past the end of the sections and could not be measured. Maximum height of the sections was 5670 µm.
[2]Width measured at widest point.
[3]Mean number per group refers to vessels only. Sample size (n) = 25 vessel strands. (4) Not applicable to the cell or tissue type.

Table 4. Comparison Between Thesis and Literature Data and Observations For *Xanthorhiza simplicissima* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Element Dimensions	249.4 µm mean length; 32.9 µm mean width	50	(1)	167.0 µm mean length; 20.0 µm mean width (Carlquist 1995 c.)
Vessel Element Perforation Plate	Simple	50	(1)	Simple (Carlquist 1995 c.)
Vessel Element Secondary Wall Deposition Pattern	Pitted	50	No annular, helical, scalariform, or reticulate patterns.	No helical vessel wall sculpturing. (Carlquist 1995 c.)
Vessel Element Secondary Wall Pitting Type	Alternating pits	50	Lateral walls are covered by numerous oval shaped pits.	Alternate circular to oval pitting. (Carlquist 1995 c.)
Vessel Number Per Group	2.5 mean vessels per group	50	Single vessels and groups lie in strands.	4.5 mean vessels per group (Carlquist 1995 c.)
Vessel Distribution	Semi-ring porous	116	Vessel strands radiate from tangentially oriented vessel clusters.	Growth rings have abundant narrow latewood vessels. (Carlquist 1995 c.)
Vessel Restriction Pattern	Vessels restricted to center of fiber area between the rays.	116	Vessels do not make contact with the rays, which they lie between.	Vessels restricted to central part of fasicular areas. No vessel contact with rays. (Carlquist 1995 c.)

Table 4. (Continued) Comparison Between Thesis and Literature Data And Observations for *Xanthorhiza simplicissima* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Storying	Largely nonstoried.	28	Some show storying. Many do not exhibit storying.	No storying. (Carlquist 1995 c.)
Ray Cell Dimensions	55.1 µm mean height; 22.4 µm mean width	54	Rays cells have an upright orientation.	Upright orientation, plus some square ray cells (no other information). (Carlquist 1995 c.)
Ray Dimensions	150.3 µm mean width (9.6 cells mean width)	26	Rays exceed the height of the sections (6000 µm) and could not be measured.	10.3 cells mean width (no other information). (Carlquist 1995 c.)
Ray Type	Multiseriate	25	(1)	Multiseriate (Paedomorphic Type II) (Carlquist 1995 c.)
Ray Distribution	Rays extend the width of the secondary xylem.	28	The xylem contains numerous rays which radiate outward like wagon wheel spokes.	Secondary xylem rays are extensions of the primary rays. (Carlquist 1995 c.)
Ray Storying	Nonstoried	25	(1)	(2)
Fiber Dimensions	351.1 µm mean length; 15.9 µm mean width	50	(1)	342.0 µm mean length (Carlquist 1995 c.)
Fiber Type	Libriform	43	(1)	Libriform (Carlquist 1995 c.)

Table 4. (Continued) Comparison Between Thesis and Literature Data And Observations for *Xanthorhiza simplicissima* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Fiber Distribution	Associated with vessels and rays.	43	Very abundant throughout the xylem.	Fibers are adjacent to rays and vessels. (Carlquist 1995 c.)
Fiber Storying	Most are nonstoried.	27	(1)	No storying in axial xylem cells. (Carlquist 1995 c.)

(1) See Character State.
(2) This information is not included in the literature.

Table 5. Comparison Between Thesis and Literature Data and Observations For *Coreopsis gigantea* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Element Dimensions	180.2 µm mean length; 51.6 µm mean width	50	(1)	210.0 µm mean length; 36.7 µm mean width (Carlquist 1985)
Vessel Element Perforation Plate	Simple	50	(1)	Simple (Carlquist 1985)
Vessel Element Secondary Wall Deposition Pattern	Helical transitional to scalariform (tending towards scalariform).	50	Helical pattern found in earlier formed xylem.	No helical vessel wall sculpturing. (Carlquist 1985)

Table 5. (Continued) Comparison Between Thesis and Literature Data And Observations for *Coreopsis gigantea* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Element Secondary Wall Pitting Type	Pseudoscalari-form pitting.	70	Lateral wall pitting does not conform to conventional pitting types.[2]	(3)
Vessel Number Per Group	1.7 mean vessels per group	26	(1)	2.2 mean vessels per group (Carlquist 1985)
Vessel Distribution	Semi-ring porous	26	Semi-ring porous patterns are seen more easily in older stems. Vessel diameter may be only marginally larger.	Semi-ring porous (Carlquist 1985)
Vessel Restriction Pattern	The concept of restriction pattern does not apply since xylary rays do not exist.	26	The vessels are part of vascular bundles which are separated by interfasicular parenchyma regions.	(3)
Vessel Storying	Vessels tend to show storying.	20	(1)	(3)
Interfasicular Parenchyma Cell Dimensions[4]	113.2 µm mean height; 69.5 µm mean width	50	Interfasicular parenchyma cells in recently formed xylem in older stems have an upright orientation.	(3)

Table 5. (Continued) Comparison Between Thesis and Literature Data And Observations for *Coreopsis gigantea* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Interfasicular Parenchyma Tissue (Interfasicular region)[4]	134.5 µm mean width (mean of 5.6 parenchyma cells)	20	Interfasicular regions exceed the height of the sections (8000 µm) and could not be measured.	(3)
Ray Type	No true xylary rays were observed. The interfasicular regions are multiseriate.	20	(1)	(3)
Ray Distribution	No true xylary rays were observed. The interfasicular regions parallel the vascular bundles.	65	(1)	(3)
Ray Storying	No true xylary rays were observed. The interfasicular regions are nonstoried.	20	(1)	(3)
Fiber Dimensions	336.7 µm mean length; 27.1 µm mean width	53	(1)	(3)
Fiber Type	Libriform	53	(1)	(3)

Table 5. (Continued) Comparison Between Thesis and Literature Data And Observations for *Coreopsis gigantea* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Fiber Distribution	Closely associated with vessels.	22	Fibers are not very abundant in the xylem.	(3)
Fiber Storying	Fibers tend to be storied.	22	Fibers are found only in strands, usually 2-4 fibers wide.	(3)

(1) See Character State.
[2]See pseudoscalariform lateral wall pitting in the discussion of paedomorphosis in the Background section.
(3) This information is not included in the literature.
[4]No true xylary rays or ray cells were observed in the *Coreopsis gigantea* specimens. Instead, interfasicular regions are present. Measurements were taken from parenchyma cells from interfasicular regions beside new vessels in older stems.

Table 6. Comparison Between Thesis and Literature Data and Observations For *Mahonia bealei* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Element Dimensions	257.5 µm mean length; 24.7 µm mean width	50	(1)	272.0 µm mean length; 13.0 µm mean width (lumen diameter) (Carlquist 1995 a.)
Vessel Element Perforation Plate	Simple	50	(1)	Simple (genus) (Carlquist 1995 a.)

Table 6. (Continued) Comparison Between Thesis and Literature Data And Observations for *Mahonia bealei* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Vessel Element Secondary Wall Deposition Pattern	Helical	50	(1)	Helical (Carlquist 1995 a.)
Vessel Element Secondary Wall Pitting Type	Alternate pitting	30	Lateral walls have narrow oval shaped pits.	(2)
Vessel Number Per Group	6.7 mean vessels per group	25	Strands consist of both solitary vessels and vessel clusters.	11.8 mean vessels per group (Carlquist 1995 a.)
Vessel Distribution	Semi-ring porous	26	Vessel groups within the strands tend to be diagonally oriented as the strand zig zags between the two rays.	Semi-ring porous (genus) (Carlquist 1995 a.)
Vessel Restriction Pattern	Vessels make frequent contact with both rays.	25	The vessel strands lie between two rays.	Diagonally oriented vessel groups are not restricted to the center of the fasicular area (Carlquist 1995 a.)
Vessel Storying	Some vessels show storying. Many do not show storying.	25	(1)	At least some vessels are storied. (Carlquist 1995 a.)

Table 6. (Continued) Comparison Between Thesis and Literature Data And Observations for *Mahonia bealei* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Ray Cell Dimensions (All)	28.9 µm mean height; 40.3 µm mean width	50	Rays consist of a mix of both upright and procumbent oriented ray cells.	Rays contain both upright and procumbent ray cells. (Carlquist 1995 a.)
Ray Cell Dimensions (subsample)	34.0 µm mean height; 31.4 µm mean width (upright orientation)	20	Some of the sampled ray cells were upright in orientation.	Rays contain both upright and procumbent ray cells. (Carlquist 1995 a.)
Ray Cell Dimensions (subsample)	25.5 µm mean height; 46.1 µm mean width	30	Some of the sampled ray cells were procumbent in orientation.	Rays contain both upright and procumbent ray cells. (Carlquist 1995 a.)
Ray Dimensions	63.8 µm mean width (3.7 cells mean width)	27	Rays exceed the height of the sections (5670 µm) and could not be measured.	4.4 cells mean width (only information available) (Carlquist 1995 a.)
Ray Type	Multiseriate predominantly, with a few uniseriate rays	27	(1)	Multiseriate (Carlquist 1995 a.)
Ray Distribution	Rays extend the width of the secondary xylem.	25	The xylem contains numerous rays which radiate outwards like wagon wheel spokes.	(2)
Ray Storying	Nonstoried	27	(1)	Nonstoried (Carlquist 1995 a.)

Table 6. (Continued) Comparison Between Thesis and Literature Data And Observations for *Mahonia bealei* Xylem

Character	Character State	Thesis Sample Size	Thesis Observations	Literature Data and Observations
Fiber Dimensions	387.1 µm mean length; 15.5 µm mean width	50	(1)	541.0 µm mean length (only information available) (Carlquist 1995 a.)
Fiber Type	Libriform	50	(1)	Libriform (genus) (Carlquist 1995 a.)
Fiber Distribution	Associated with vessels and rays.	29	Very abundant throughout the xylem.	Vessel groups are embedded in fibers. (Carlquist 1995 a.)
Fiber Storying	Some limited storying occurs. Most are nonstoried.	25	(1)	No storying in libriform fibers. (Carlquist 1995 c.)

(1) See Character State.
(2) This information is not included in the literature.

Table 7. Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coptis	Herbaceous Herbaceous (*Coptis trifolia, Coptis laciniata, Coptis groenlandia*)	Kim et al. 2004 Kartesz 2008; Halpern 1989; Bratton 1976	Perennial (*Coptis trifolia)*	Kartesz 2008	Stem height 7.6-15.2 cm Small stature herb	Novak and Lemmon 2005 Halpern 1989	Very low.
Xanthorhiza	Woody Woody shrub or subshrub	Kim et al. 2004 Isnard et al. 2003	Perennial	Isnard et al. 2003	The wood is like that of a small shrub. It produces weakly upright woody stems. It grows no more than 60 cm tall.	Carlquist 1995 c. Isnard et al. 2003	Very high (confirmed through thesis research).

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Ranunculus	Herbaceous	Kim et al. 2004; Judd et al. 2002; Hoot 1991; Stebbins 1938	Perennial	Hoot 1991	*Ranunculus repens* is a creeping low growing perennial herb with prostrate to nearly erect stems (20-30 cm tall).	Enkerlin 2008	Low to moderate.
	Herbaceous (*Ranunculus repens; Ranunculus longirostris, Ranunculus hispidus*)	Enkerlin 2008; Kartesz 2008	Perennial (*Ranunculus repens; Ranunculus longirostris, Ranunculus hispidus*) Genus has both annual and perennial species.	Enkerlin 2008; Kartesz 2008 Stebbins 1938	*Ranunculus longirostris* is a semi-erect perennial herb (30.5 cm tall). *Ranunculus hispidus* is a perennial herb with a single erect stem (76.2 cm tall).	Kartesz 2008	

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Hydrastis	Herbaceous Herbaceous (*Hydrastis canadensis*)	Kim et al. 2004 Sinclair and Catling 2001	Perennial	Sinclair and Catling 2001	*Hydrastis canadensis* is a herbaceous perennial with a stem 30 cm tall that grows in mesic woods.	Sinclair and Catling 2001	Low to moderate.
Glaucidium	Herbaceous Herbaceous (*Glaucidium palmatum*)	Kim et al. 2004 Kumazawa 1930	Perennial	Kumazawa 1930	*Glaucidium palmatum* is a herbaceous perennial (45-60 cm tall).	Kumazawa 1930	Low to moderate.
Caulophyllum	Herbaceous Herbaceous (*Caulophyllum thalictroides*)	Kim et al. 2004; Meachum 1980 Kartesz 2008; Kim and Jansen 1998	Perennial (*Caulophyllum thalictroides*)	Kartesz 2008	*Caulophyllum thalictroides* is an errect herbaceous perennial with a multiple stem growth form (76.2 cm tall).	Kartesz 2008	Moderate.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Nandina	Woody Shrub	Scheper 2008; Kim et al. 2004; Kim and Jansen 1998 Pernas and Bergeron 2008; Scheper 2008; Loconte and Estes 1989	Evergreen perennial	Pernas and Bergeron 2008; Scheper 2008	*Nandina domestica* is an evergreen shrub that grows 180 cm tall. It is found in multistemmed clumps, and has compound alternate leaves. *Nandina domestica* is a woody evergreen shrub with multiple nonbranching stems (180-240 cm tall).	Pernas and Bergeron 2008 Scheper 2008	Very high.
Menispermum	Woody Vine (*Menispermum canadense*)	Kim et al. 2004; Stebbins 1938; Myers 1924 Kartesz 2008	Perennial	Kartesz 2008	The stem of *Menispermum canadense* contains 8 vascular bundles. *Menispermum canadense* is a vine or liana twining to 5 m around trees.	Dormer 1954 Myers 1924	Very high.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Tinospora	Woody Woody (*Tinospora caffra*; *Tinospora siamensis*)	Kim et al. 2004 Senbeta et al. 2005; Forman 1988	Perennial plant	Forman 1988	*Tinospora caffra* is a liana which twines around its host. *Tinospora siamensis* is a slender woody climber up to 10 m tall. Its glabrous stems are up to 2 cm in diameter. The genus is found in disturbed forests.	Senbeta et al. 2005 Forman 1988	Very high.
Akebia	Woody Woody (*Akebia quinata*)	Kim et al. 2004 Swearingen et al. 2006	Perennial (*Akebia quinata*)	Swearingen et al. 2006	*Akebia quinata* is a woody perennial plant that grows as either a climbing vine, or as ground cover. It is deciduous in cooler regions, but evergreen in warmer regions. The stem of *Akebia quinata* contains 5 vascular bundles.	Swearingen et al. 2006 Dormer 1954	Very high.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Sinofranchetia	Woody Vine	Gianoli 2004; Kim et al. 2004 Loconte and Estes 1989	Perennial plant	Marsh 2008	*Sinofranchetia chinensis* is a deciduous climber that twines around other plants. It grows 15 m tall. *Sinofranchetia sinensis* stems have 31 vascular bundles.	Marsh 2008 Dormer 1954	Very high.
Decaisnea	Woody Woody (*Decaisnea fargesii*) Shrub	Kim et al. 2004; Stebbins 1938 Bancroft 1930 Loconte and Estes 1989	Perennial plant	Marsh 2008	*Decaisnea fargesii* is a deciduous shrub which grows 4 m tall. The stem of *Decaisnea fargesii* contains 33 vascular bundles.	Marsh 2008 Dormer 1954	Very high.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Circaeaster	Herbaceous Herbaceous (*Circaeaster agrestis*)	Kim et al. 2004; Takhtajan 1997; Loconte and Estes 1989 eFloras 2008	Species of the genus may be perennial or annual.	Takhtajan 1997	*Circaeaster agrestis* is an herb 3-10 cm tall.	eFloras 2008	Very low.
Kingdonia	Herbaceous	Kim et al. 2004; Laconte and Estes 1989; Balfour and Smith 1914	Perennial	Balfour and Smith 1914	*Kingdonia uniflora* is a glabrous plant with a flowering stem 7-12 cm tall. The stem has one basal leaf.	Balfour and Smith 1914	Low.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Dicentra	Herbaceous Herbaceous (*Dicentra cullaria*, *Dicentra spectabilis*) Geophyte	Kim et al. 2004 eFloras 2008; Kartesz 2008; Kopp 2008 Walton and Hufford 1994	Perennial (*Dicentra cullaria*, *Dicentra spectabilis*)	eFloras 2008; Kopp 2008	*Dicentra cullaria* is a perennial with a scapose stalk. *Dicentra spectabilis* is an herbaceous perennial 30.5-91.4 cm tall.	eFloras 2008 Kopp 2008	Low.
Pteridophyllum	Herbaceous Herbaceous (*Pteridophyllum racemosum*)	Kim et al. 2004; Kadereit et al. 1994 Tani et al. 2001	Perennial Perennial or biennial	Tani et al. 2001 Kadereit et al. 1994	*Pteridophyllum racemosum* is an evergreen perennial herb found in the understory of mature trees.	Tani et al. 2001	Low.

Table 7. (Continued) Determination of Woodiness in Ranunculales Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Hypecoum	Herbaceous (*H. imberbe, H. pendulum)*	eFloras 2008; Kartesz 2008; Kim et al. 2004	Annual (*H. imberbe, H. pendulum)*	eFloras 2008 Kartesz 2008	*Hypecoum leptocarpum* is a procumbent to erect annual, profusely branched at the base. The stem is 15-30 cm tall, with basal leaves.	eFloras 2008	Low to moderate.
Euptelea	Woody Decidous tree (*Euptelea polyandra, Euptelea pleiosperma*) Tree or shrub *(Euptelea polyandra*)	Kim et al. 2004 Marsh 2008; Sakai et al. 1995 Garrison 1949	Perennial plant	Marsh 2008	*Euptelea polyandra* is a deciduous tree (8 m tall). *Euptela pleiosperma* is a decidous tree (9 m tall).	Marsh 2008	Very high.

Table 8. Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis douglasii	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Small (6-20 cm tall) annual with leaves entirely basal.	Smith 1984	Very low.
Coreopsis californica	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Annual (10-30 cm tall) with leaves entirely basal.	Smith 1984	Low.
Coreopsis stillmanii	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Small (8-20 cm tall) annual with leaves mostly or entirely basal.	Smith 1984	Very low.
Coreopsis bigelovii	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Annual (10-35 cm tall) with leaves mostly basal. Herbaceous annual, some-what fleshy stemmed.	Smith 1984 Smith 1975	Low to moderate.

Table 8. (Continued) Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis hamiltonii	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Small (8-20 cm tall) annual with leaves basal. Herbaceous annual, some-what fleshy stemmed.	Smith 1984 Smith 1975	Low to moderate.
Coreopsis calliopsidea	Herbaceous	Jansen et al. 1987; Smith 1975	Annual	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Annual (8-40 cm tall). Herbaceous annual, some-what fleshy stemmed.	Smith 1984 Smith 1975	Low to moderate.
Coreopsis gigantea	Herbaceous	Jansen et al. 1987; Smith 1975	Perennial	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Perennial with stout thick stems and large dissected leaves.	Crawford and Whitkus 1988	Very high (confirmed through thesis research).

Table 8. (Continued) Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis gigantea (continued)	(See previous page.)	(See previous page.)	(See previous page.)	(See previous page.)	Large (1-2 m tall) fleshy stemmed (stems 4-10 cm in diameter) perennial. Herbaceous perennial, fleshy stemmed.	Smith 1984 Smith 1975	Very high (confirmed through thesis research).
Coreopsis maritima	Herbaceous	Jansen et al. 1987; Smith 1975	Perennial	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Perennial with stout thick stem. Smaller (.3-.8 m) perennial, with hollow stems (1-2 cm in diameter). Herbaceous perennial, fleshy stemmed.	Crawford and Whitkus 1988 Smith 1984 Smith 1975	High. The plant is in the same section as *C. gigantea* (Tuckermannia). Synthetic hybrids between *C. maritima* and *C. gigantea* are highly fertile, and the species may actually a semispecies (Crawford and Whitkus 1988).

Table 8. (Continued) Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis petrophila	Woody Fruticose	Jansen et al. 1987 Smith 1975	Perennial	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987	Stems 30-150 cm tall, profusely branched, glabrate. It is in section Pseudo-Agarista: these plants are suffruticose to fruticose. Fruticose small shrub.	Crawford 1976 Crawford 1976 Smith 1975	Very high.
Coreopsis senaria	Suffruticose to fruticose.	Crawford 1976	Perennial	Archibald et al. 2005; Mort et al. 2004	Species is in section Pseudo-Agarista: these plants are suffruticose to fruticose.	Crawford 1976	Very high.
Coreopsis mutica	Woody Suffruticose	Jansen et al. 1987 Sherff 1936	Perennial	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987	Large shrub over 3 m tall, with most individuals 1-3 m tall.	Crawford 1982	Very high.

Table 8. (Continued) Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis mutica (continued).	(See previous page.)	(See previous page.)	(See previous page.)	(See previous page.)	Small shrub. Species is in section Electra: these plants are suffruticose to fruticose. Species is in section Electra: these plants are suffruticose.	Smith 1975 Crawford 1976 Sherff 1936	Very high.
Coreopsis cyclocarpa	Woody Herbaceous	Jansen et al. 1987 Smith 1975	Perennial	Archibald et al. 2005; Mort et al. 2004; Jansen et al. 1987; Smith 1975	Suffrutescent perennial. He states it is a woody perennial. Herbaceous with several stems from the base. Herbaceous perennial	Crawford 1981 Smith 1975 Sherff 1936	High.

Table 8. (Continued) Determination of Woodiness in *Coreopsis* Species

Species	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Coreopsis cyclocarpa (continued).	(See previous page.)	(See previous page.)	(See previous page.)	(See previous page.)	Species is in section Anathysana: these plants are suffrutescent. Species is in section Anathysana: these plants are herbaceous perennials.	Crawford 1976 Sherff 1936	High.
Dahlia coccinea (outgroup)	Herbaceous	Vivar-Evans et al. 2006	Perennial	Archibald et al. 2005: Mort et al. 2004	Tuberous perennial. Frost tender tuberous perennial, .9-1.5 m tall. Stems are slender and branched. Perennial herbaceous wild plant with tuberous roots.	Harris 2008 Saylor 2008 Vivar-Evans et al. 2006	Low to moderate.

Table 9. Determination of Woodiness in Berberidaceae Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Vancouveria	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Kartesz 2008	*Vancoveria hexandra* is a perennial with long basal stems (20.3-40.6 cm tall). It has pinnately compound deciduous leaves.	Turner and Gustafson 2006	Low.
Epimedium	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Kang 1990	*Epimedium koreanum* is a low rhizomatous perennial herb that grows to 30 cm height.	Kang 1990	Low.
Achlys	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980; Fukada 1967	Perennial	Fukada 1967	Both *Achlys triphylla* and *Achlys japanica* are perennial herbs with branching, creeping rhizomes. *Achlys californica* is a slender erect perennial herb that grows to 39 cm height. It has branched, creeping rootstocks.	Fukada 1967 Fukada and Baker 1970	Low.

Table 9. (Continued) Determination of Woodiness in Berberidaceae Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Sinopodophyllum	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989	Perennial	Xiao et al. 2006	*S. hexandrum* is an endangered perennial herb found in Asia.	Xiao et al. 2006	Low.
Diphylleia	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Glazer 2008	*Diphylleia* has stems with scattered vascular bundles. *Diphylleia cymosa* is a rhizomatous herb 40-100 cm tall.	Judd et al. 2002 Glazer 2008	Very low.
Dysosma	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Woodson 1928	*Dysosma pleiantha* is an herbaceous caulescent perennial.	Woodson 1928	Low to moderate.
Podophyllum	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial (*Podophyllum peltatum*)	Jones and Watson 2001	*Podophyllum* has stems with scattered vascular bundles.	Judd et al. 2002	Very low.

Table 9. (Continued) Determination of Woodiness in Berberidaceae Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Plagiorhegma	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Marsh 2008	*Plagiorhegma dubia* (AKA *Jeffersonia dubia*) is a perennial that grows to 20 cm height.	Marsh 2008	Low.
Jeffersonia	Herbaceous	Kim and Jansen 1998; Baskin and Baskin 1989; Laconte and Estes 1989; Meachum 1980	Perennial (*Jeffersonia diphylla)*	Judziewicz and Freire 2008; Baskin and Baskin 1989	*Jeffersonia diphylla* is an erect perennial that grows 10.2-20.3 cm tall. *Jeffersonia diphylla* is an herbaceous perennial found in mesic forests.	Judziewicz and Freire 2008 Baskin and Baskin 1989	Low.
Ranzania	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Schmid 2002	Small rhizomatous perennial that grows 30.5 cm tall (*Ranzania japonica* AKA *Podophyllum japonicum*).	Schmid 2002	Low.

Table 9. (Continued) Determination of Woodiness in Berberidaceae Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Mahonia	Woody Shrub	Kim and Jansen 1998 Loconte and Estes 1989; Meachum 1980	Perennial plant	Rehder 2001	Evergreen unarmed shrubs, rarely small trees.	Rehder 2001	Very high (confirmed through thesis research).
Berberis	Woody Shrub	Kim and Jansen 1998 Loconte and Estes 1989; Meachum 1980	Perennial plant	Rehder 2001	Evergreen or deciduous spiny shrubs, rarely small trees.	Rehder 2001	Very high.
Gymnospermium	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	Hayek 2008	Small perennial tuberous herb with yellow flowers. Height of 5 cm.	Hayek 2008	Low.
Leontice	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989; Meachum 1980	Perennial	eFloras 2008	*Leontice evermannii* is a perennial herb that grows 30-50 cm tall.	eFloras 2008	Low.

Table 9. (Continued) Determination of Woodiness in Berberidaceae Genera

Genus	Habit	Source	Longevity	Source	Description	Source	Probability of Woodiness
Caulophyllum	Herbaceous	Kim and Jansen 1998; Loconte and Estes 1989	Perennial	eFloras 2008; Kartesz 2008	Genus consists of understory deciduous herbs that grow 20-90 cm tall. A perennial with multiple stems.	eFloras 2008; Kartesz 2008	Low.
Nandina	Woody Shrub.	Scheper 2008; Kim and Jansen 1989 Pernas and Bargeron 2008; Scheper 2008; Loconte and Estes 1989	Evergreen perennial	Pernas and Bargeron 2008; Scheper 2008	*Nadina domestica* is an evergreen shrub that grows 180 cm tall. It is found in multistemmed clumps with compound alternate leaves. *Nandina domestica* is a woody evergreen shrub with multiple nonbranching stems (180-240 cm tall).	Pernas and Bergeron 2008 Scheper 2008	Very high.

APPENDIX B. FIGURES

Figure 1. *Xanthorhiza simplicissima* in the field.

Figure 2. Close-up of *Xanthorhiza simplicissima* in the field.

Figure 3. *Coreopsis gigantea* in the field. (*Coreopsis* photos by Dr. Bruce Kirchoff.)

Figure 4. Close-up of *Coreopsis gigantea*

Figure 5. *Mahonia bealei* in the field.

Figure 6. Close-up of *Mahonia bealei* in the field.

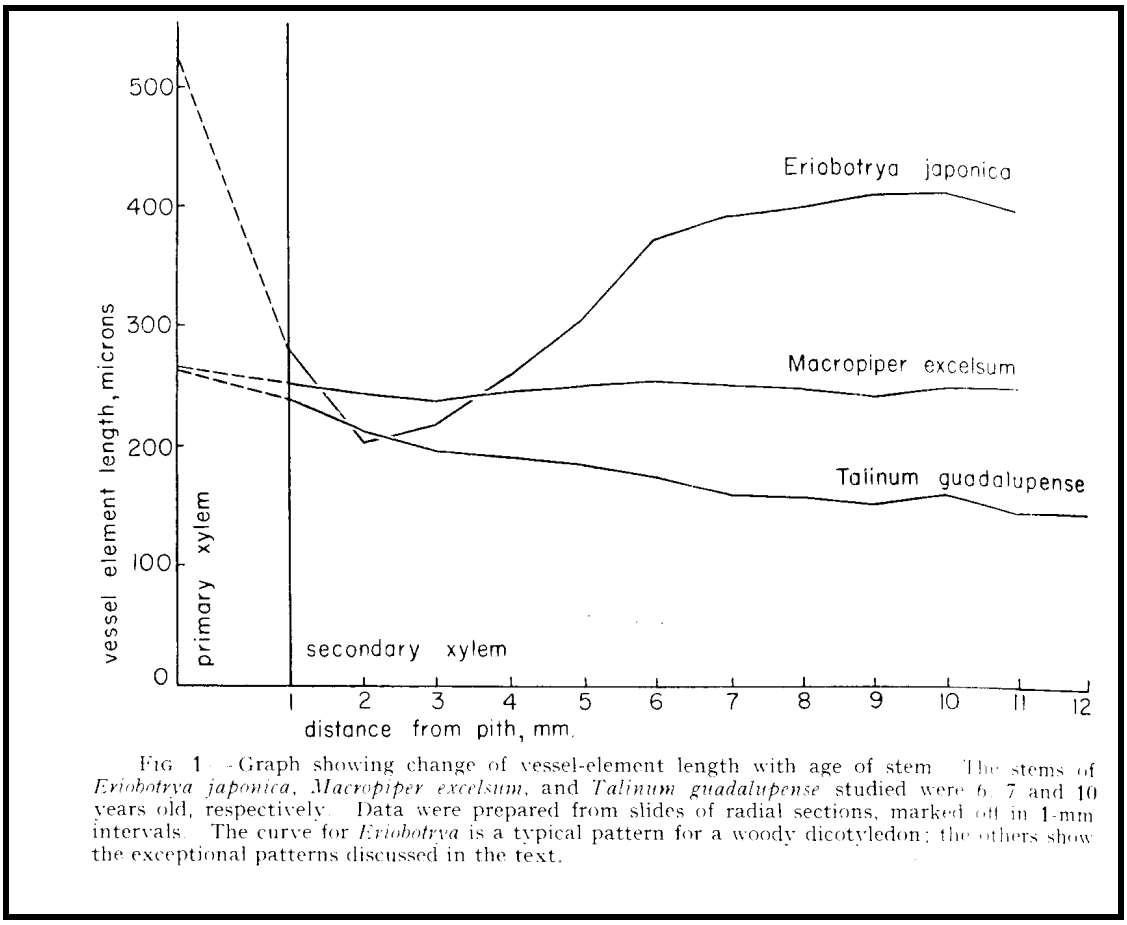

FIG. 1.—Graph showing change of vessel-element length with age of stem. The stems of *Eriobotrya japonica*, *Macropiper excelsum*, and *Talinum guadalupense* studied were 6, 7 and 10 years old, respectively. Data were prepared from slides of radial sections, marked off in 1-mm intervals. The curve for *Eriobotrya* is a typical pattern for a woody dicotyledon; the others show the exceptional patterns discussed in the text.

Figure 7. Change in vessel element length across the xylem of *Eriobotrya japonica*, *Macropiper excelsum*, and *Talinum guadalupense* (Carlquist 1962).

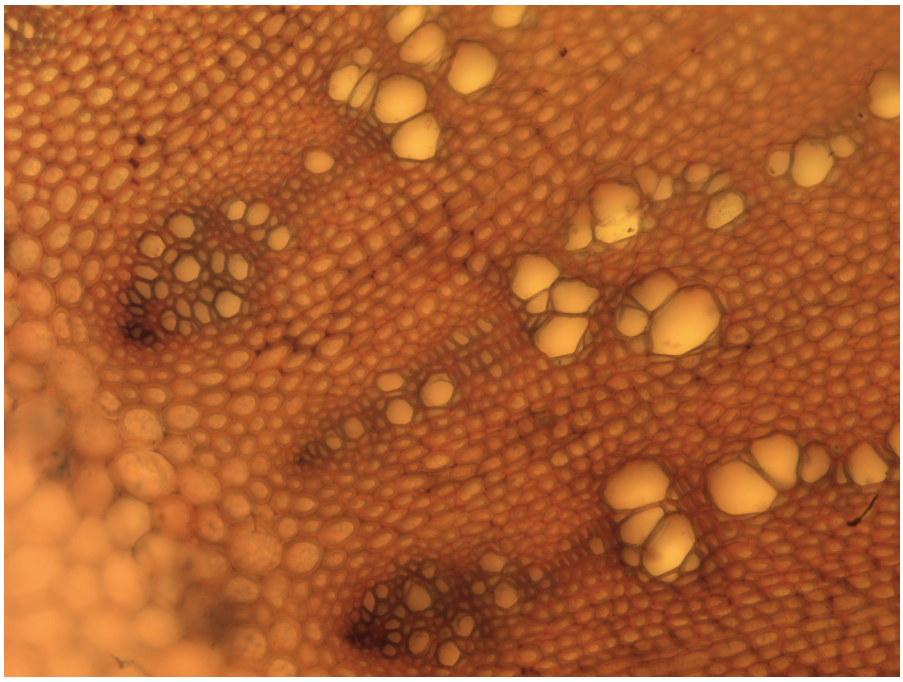

Figure 8. Transverse view of the earliest formed xylem of *Xanthorhiza simplicissima* (LM x200).

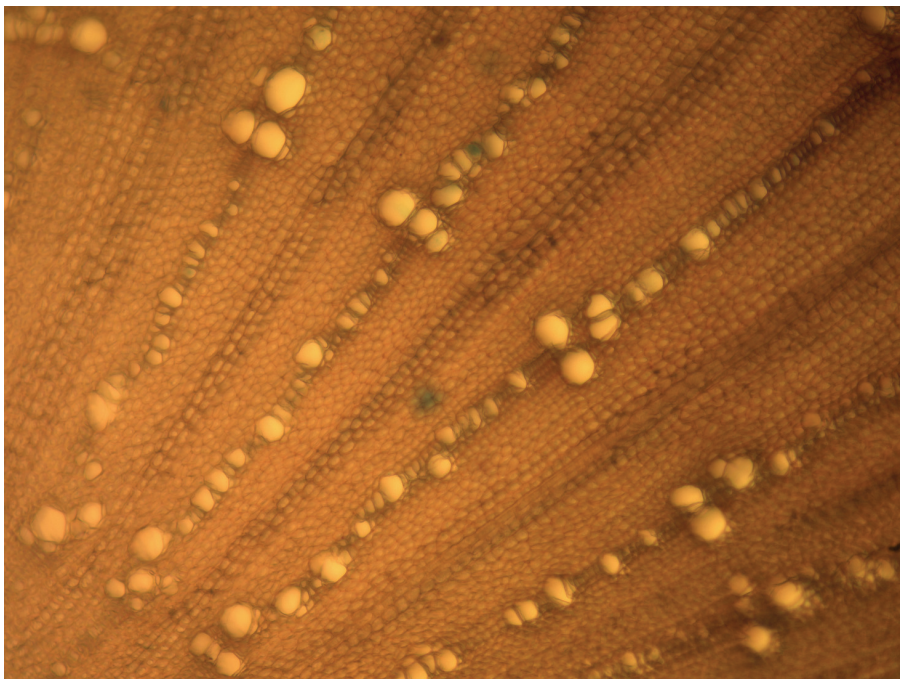

Figure 9. Transverse view of *X. simplicissima* xylem (LM x100).

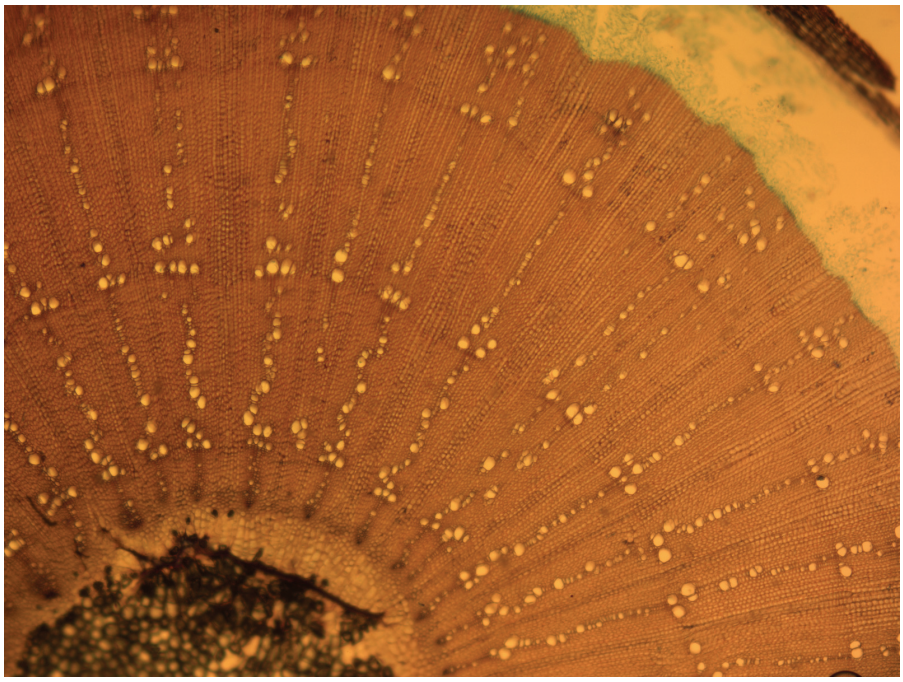

Figure 10. Transverse view of *Xanthorhiza simplicissima* stem (LM x40).

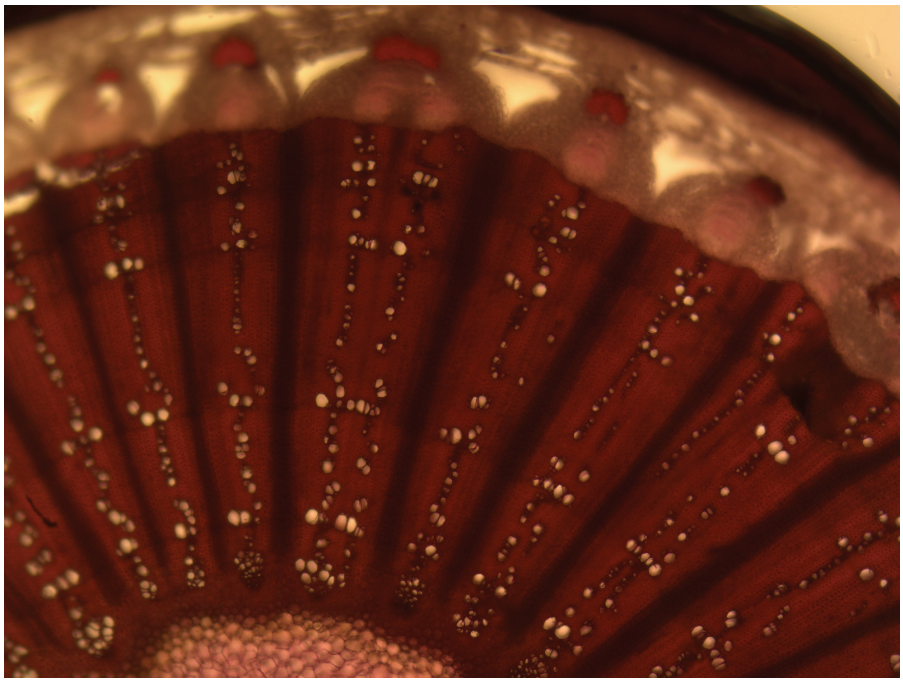

Figure 11. Transverse view of *Xanthorhiza simplicissima* stem (LM x40).

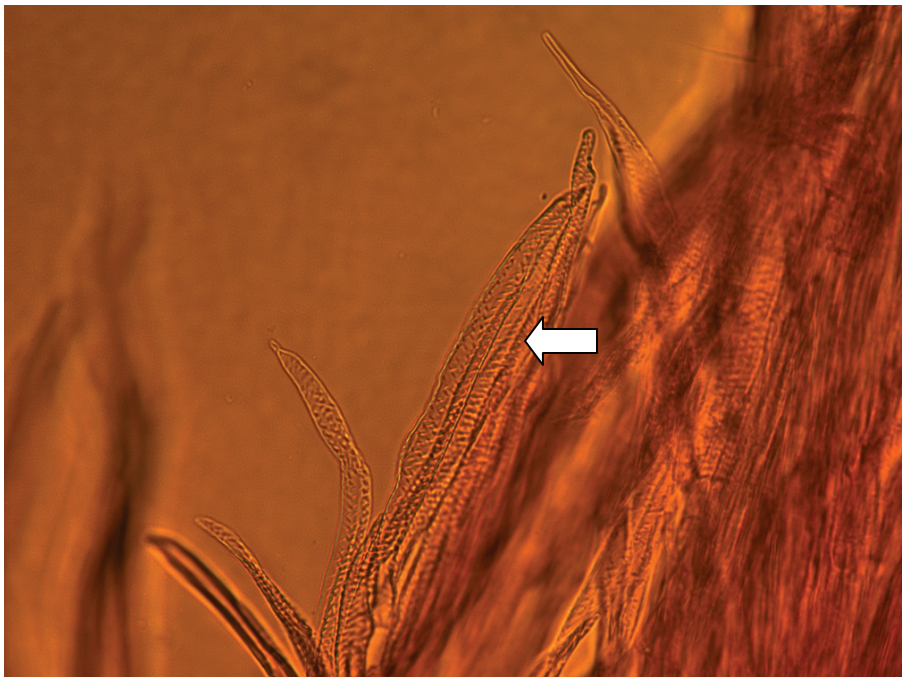

Figure 12. Tracheids (arrow) in *Xanthorhiza simplicissima* (LM x200).

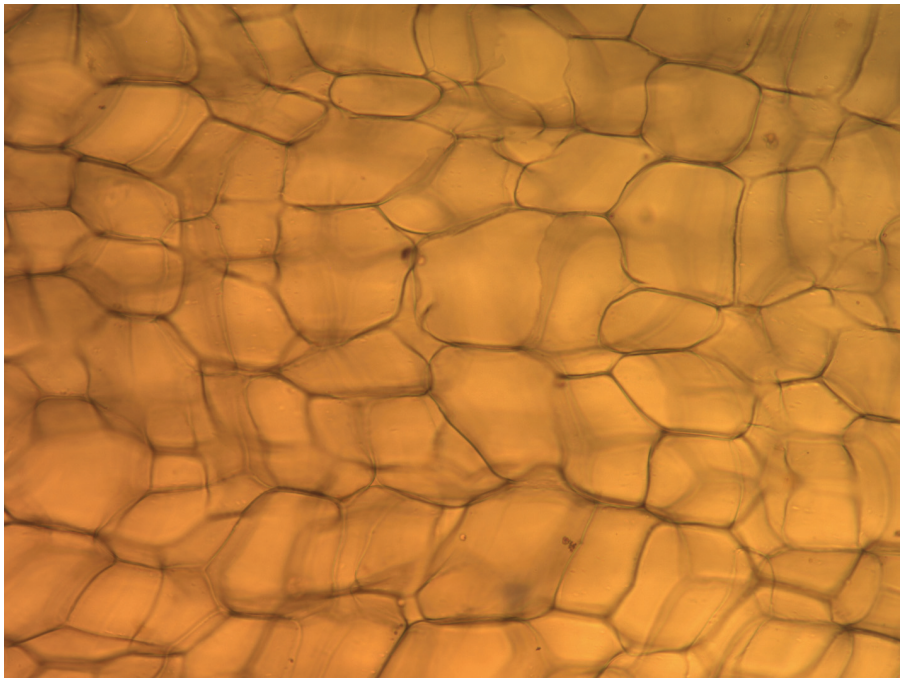

Figure 13. Tangential view of the pith cells of *Xanthorhiza simplicissima* (LM x200).

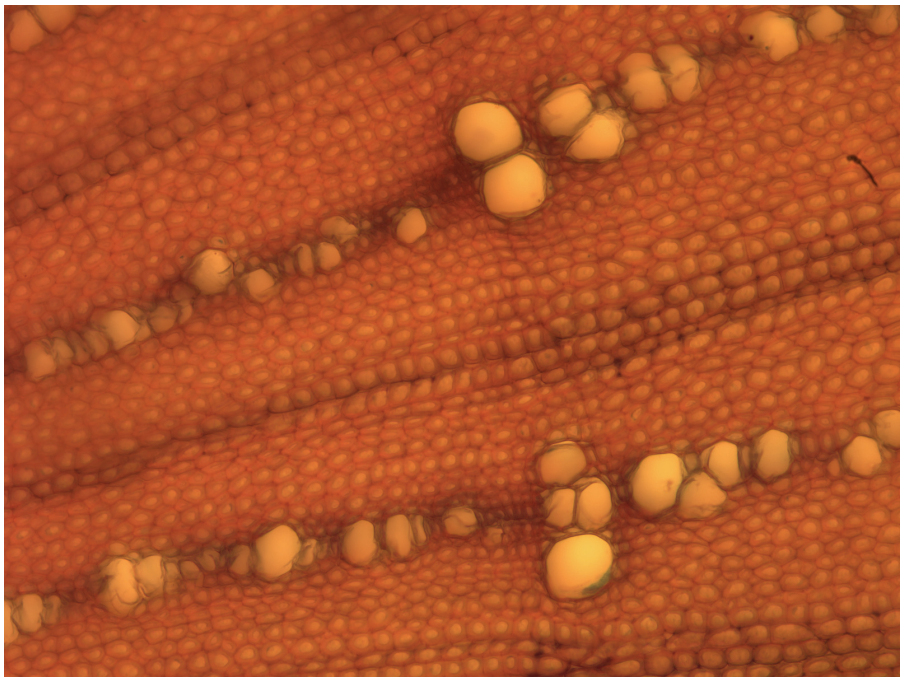

Figure 14. Transverse view of the xylem of *Xanthorhiza simplicissima* (LM x200).

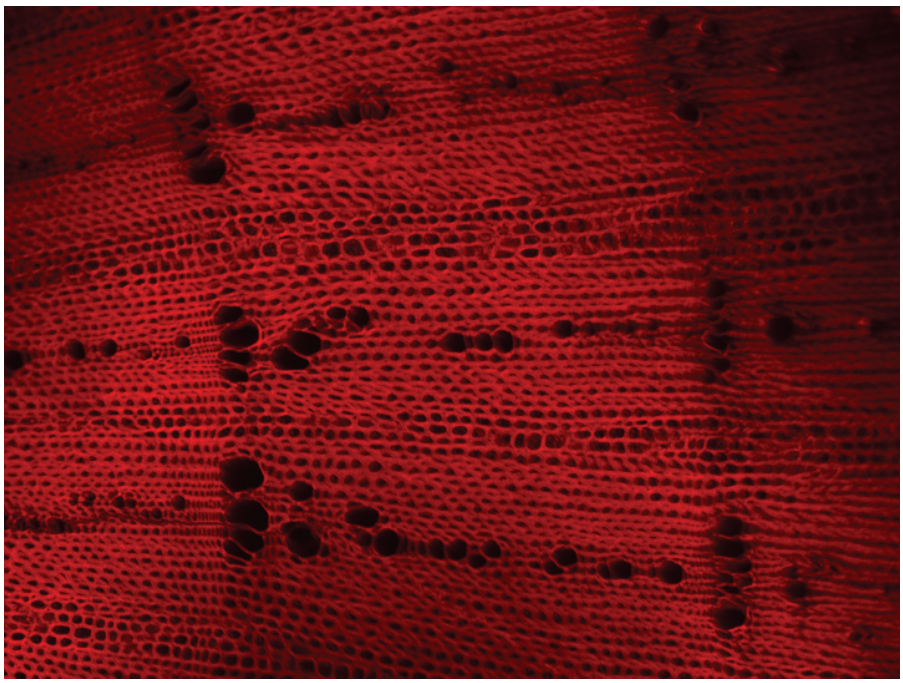

Figure 15. Growth rings in the xylem of *X. simplicissima* (CM x100).

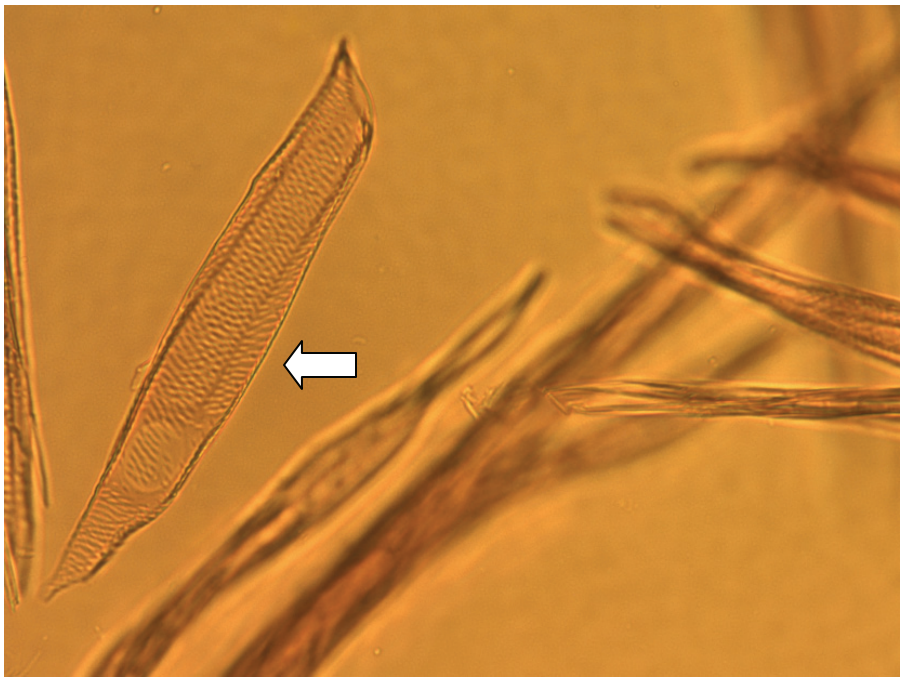

Figure 16. *Xanthorhiza simplicissima* vessel element (arrow) (LM x200).

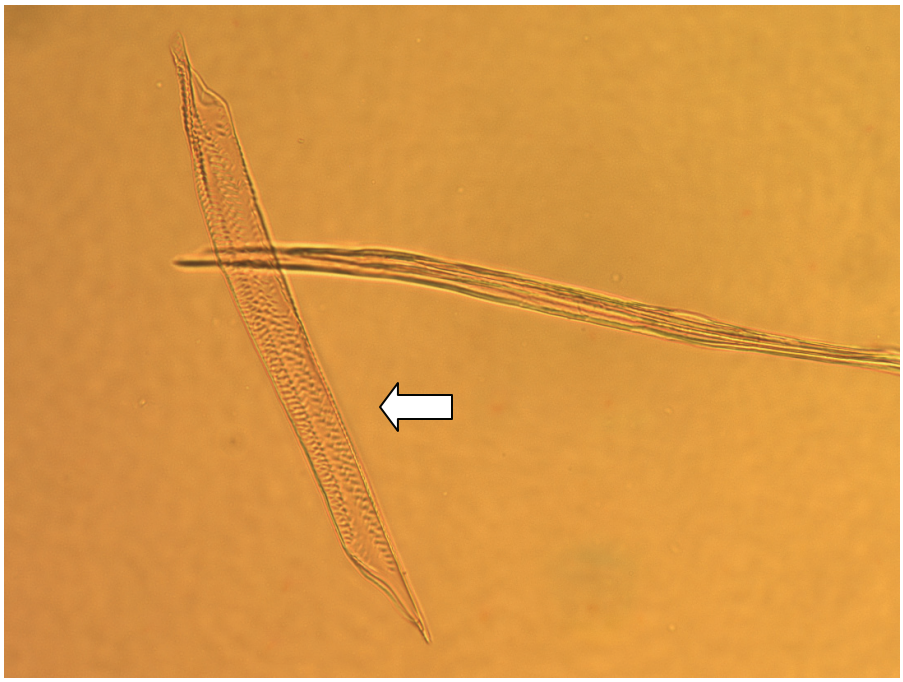

Figure 17. *Xanthorhiza simplicissima* vessel element (arrow) (LM x200).

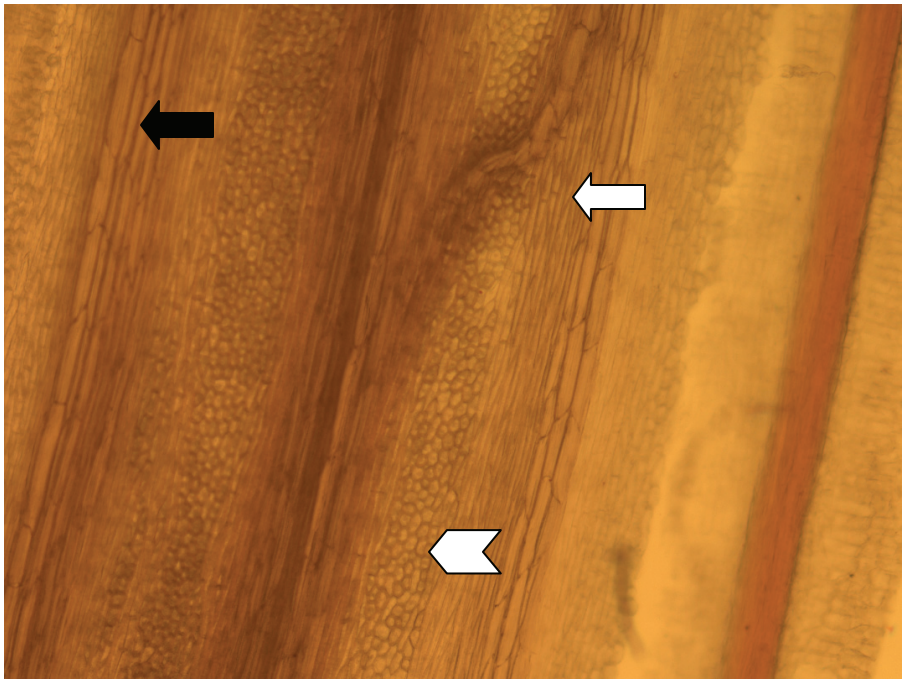

Figure 18. Tangential view of *Xanthorhiza simplicissima* xylem, showing vessels (black arrow), rays (arrowhead), and fibers (white arrow) (LM x40).

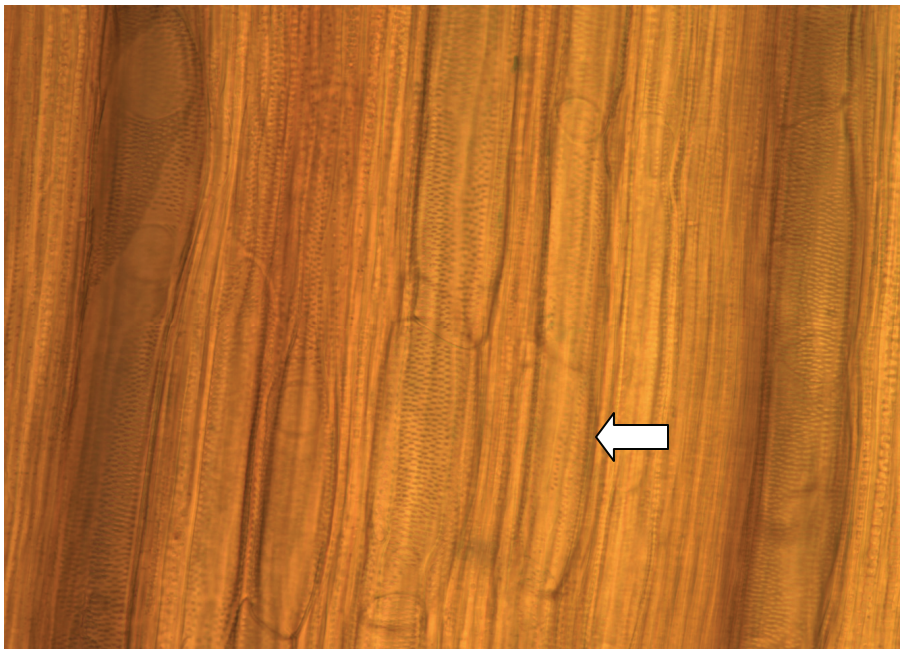

Figure 19. Radial view of *X. simplicissima* vessels (arrow) (LM x200).

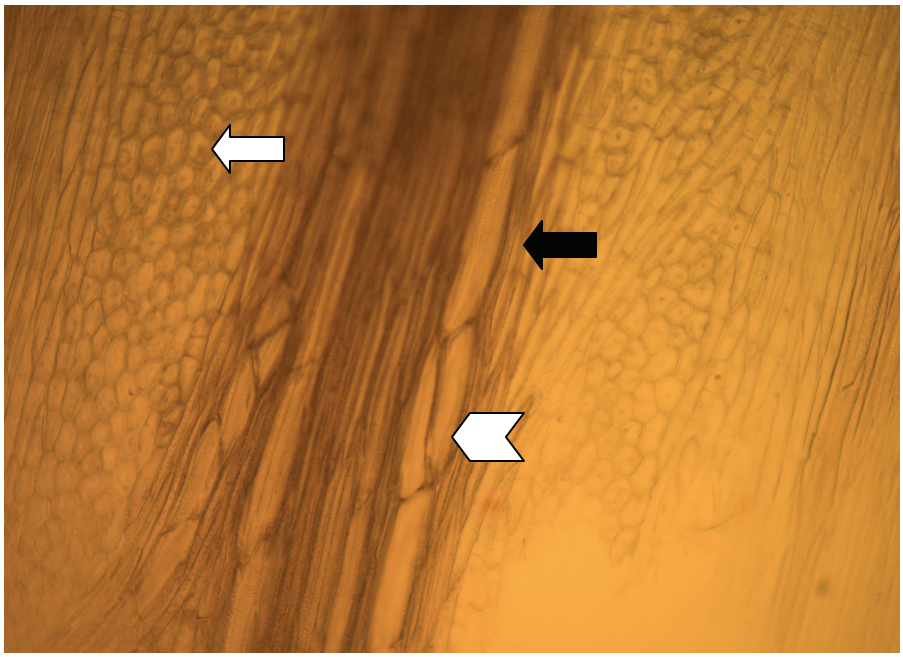

Figure 20. Tangential view of *Xanthorhiza simplicissima* rays (white arrow), vessels (arrowhead) and fibers (black arrow) (LM x100).

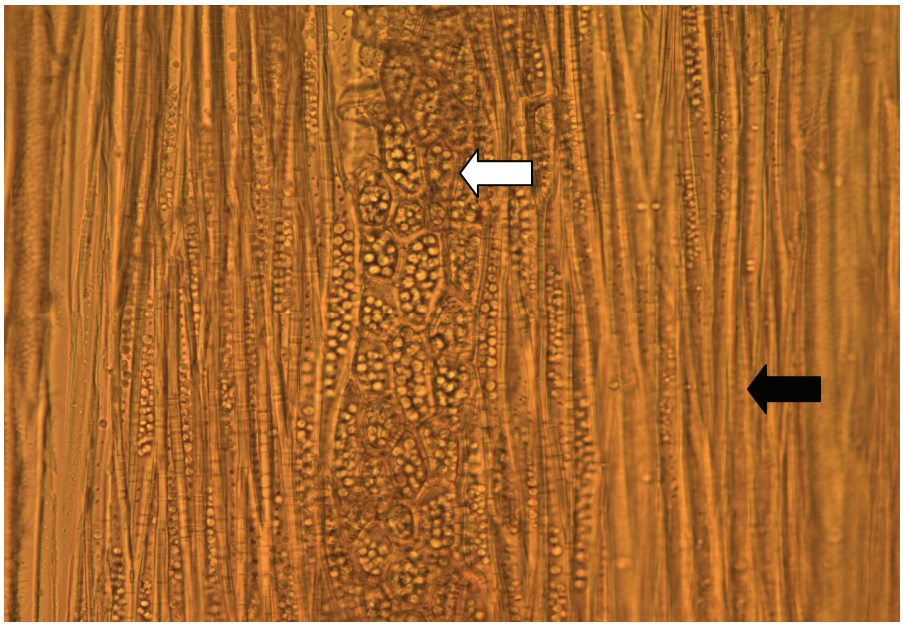

Figure 21. Tangential view of *X. simplicissima* ray (white arrow) and fibers (black arrow) (LM x200).

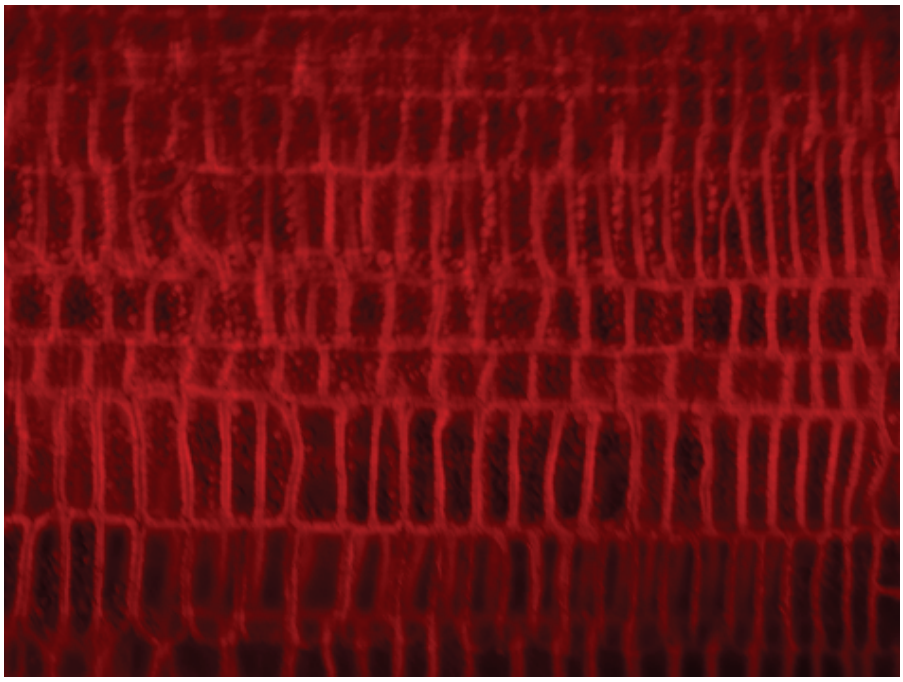

Figure 22. Radial view of *Xanthorhiza simplicissima* ray cells (CM x100).

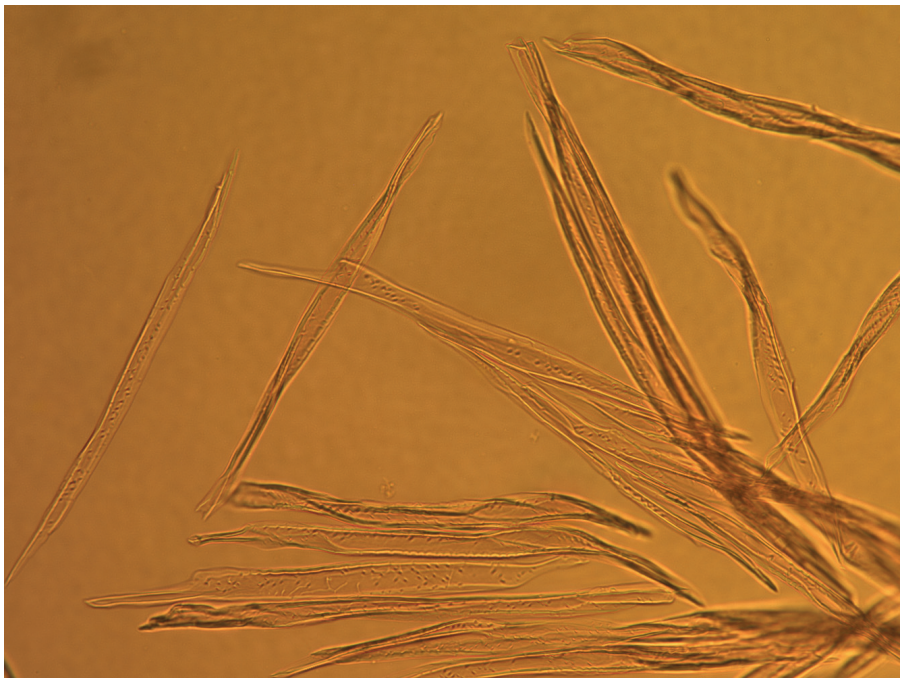

Figure 23. *Xanthorhiza simplicissima* libriform fibers (LM x200).

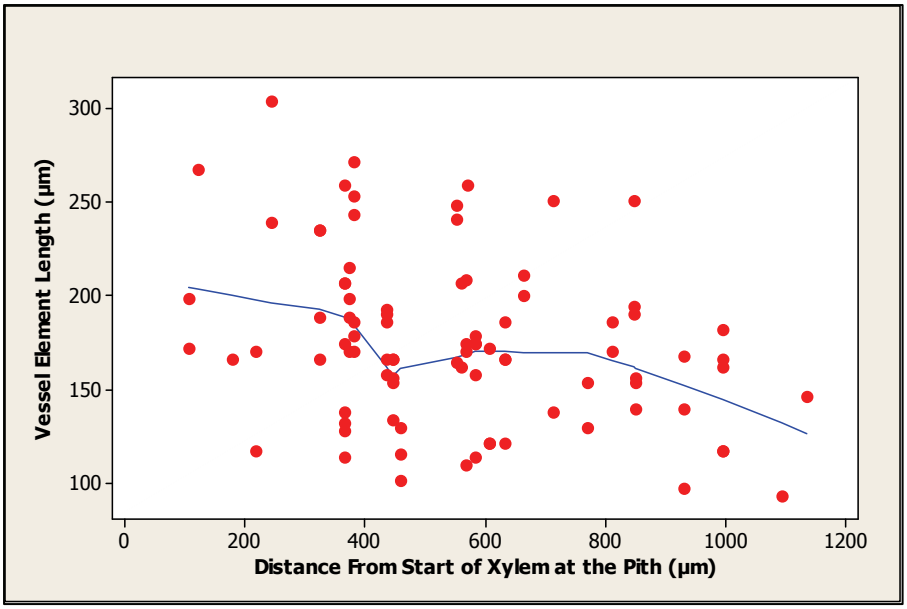

Figure 24. Change in vessel element length across the xylem of *Xanthorhiza simplicissima* (Locally Weighted Scatterplot Smoother line applied).

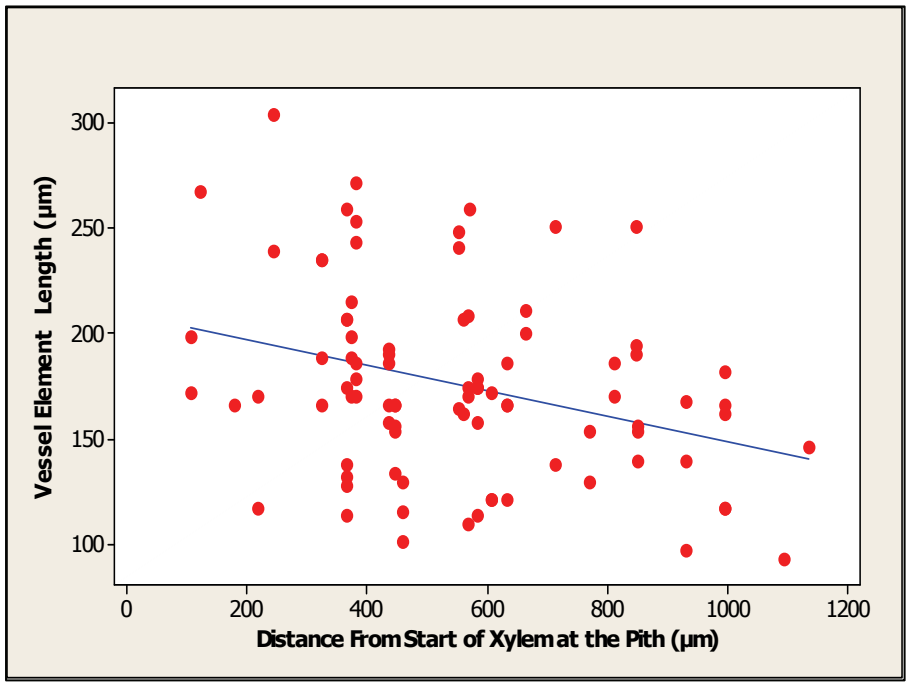

Figure 25. Change in vessel element length across the xylem of *X. simplicissima* (linear regression line fitted).(p=.002; r^2=10.4%)

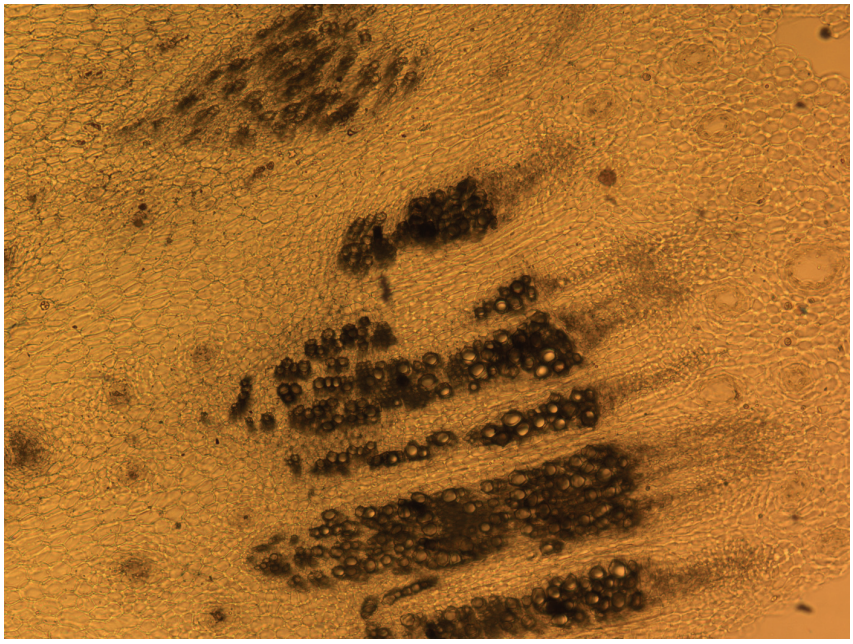

Figure 26. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x40).

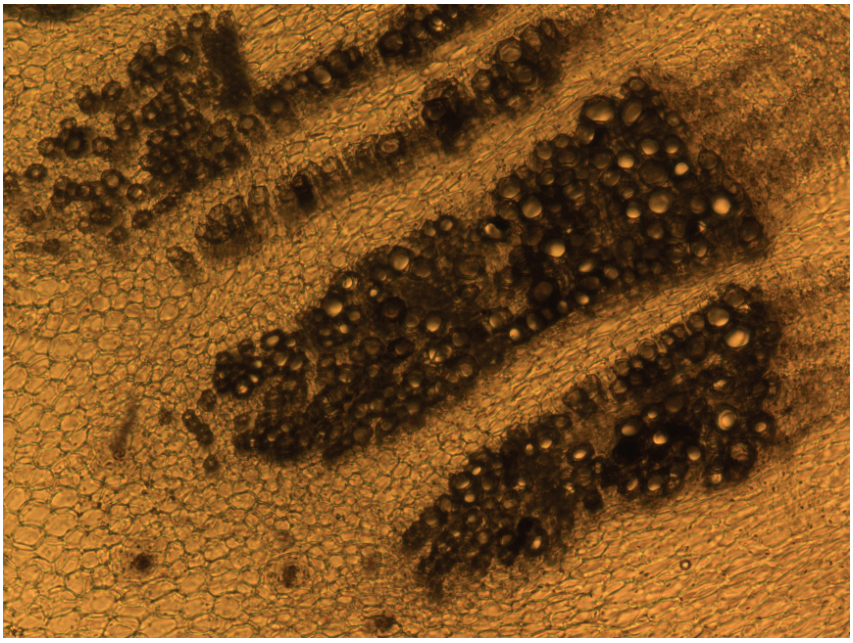

Figure 27. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x100).

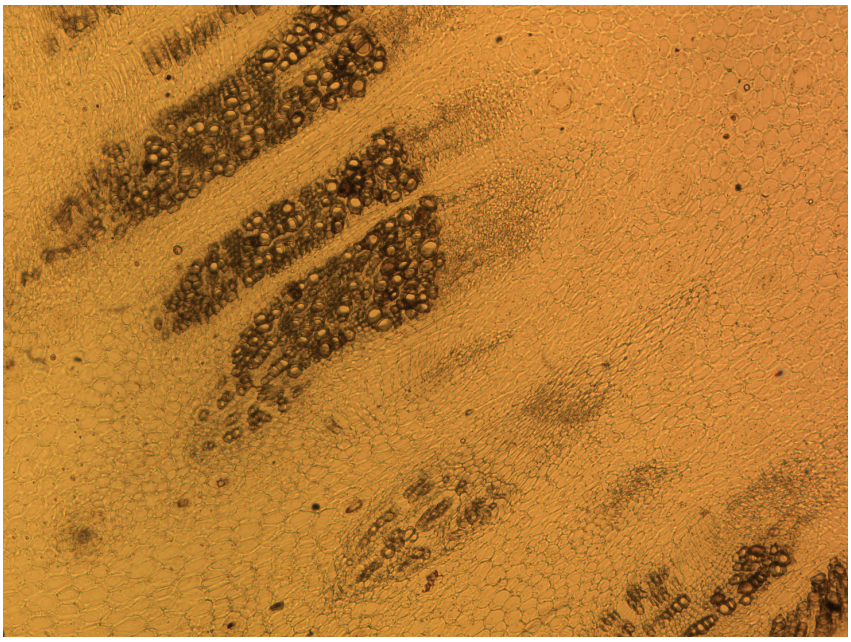

Figure 28. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x40).

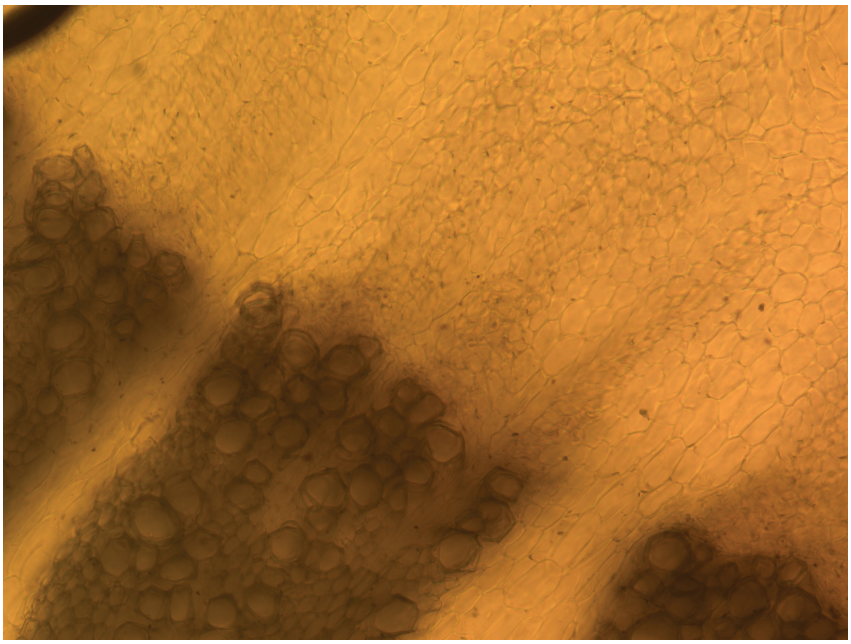

Figure 29. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x100).

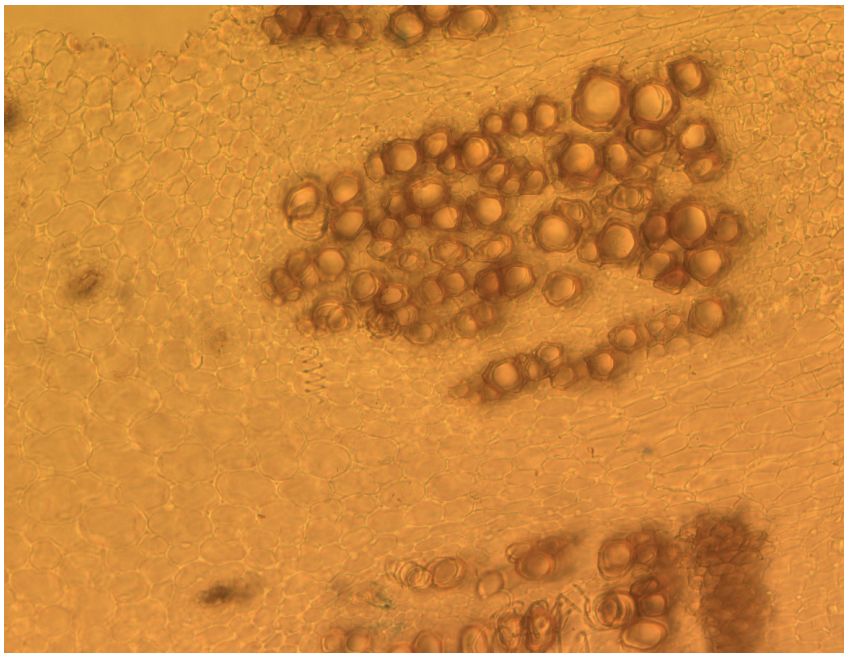

Figure 30. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x100).

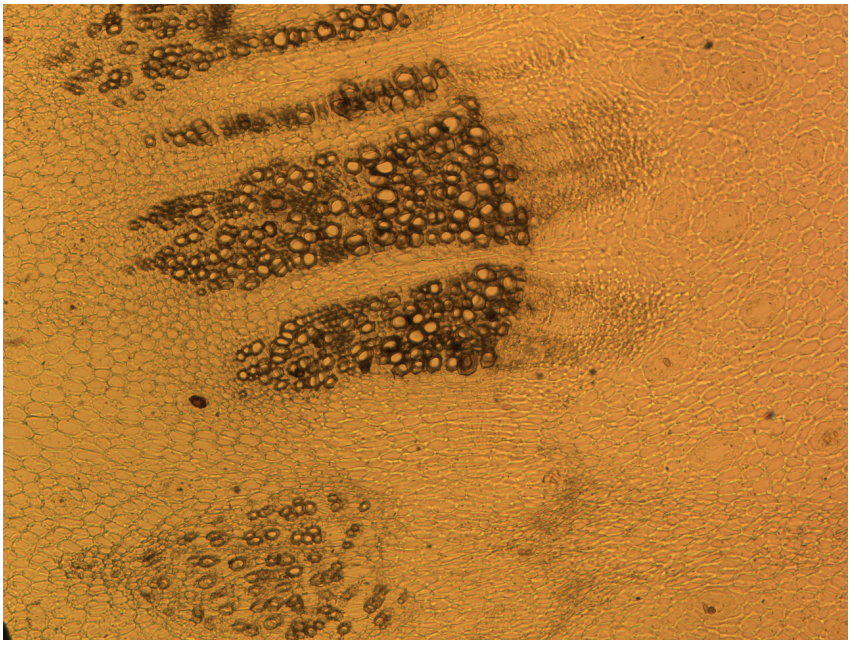

Figure 31. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x 40).

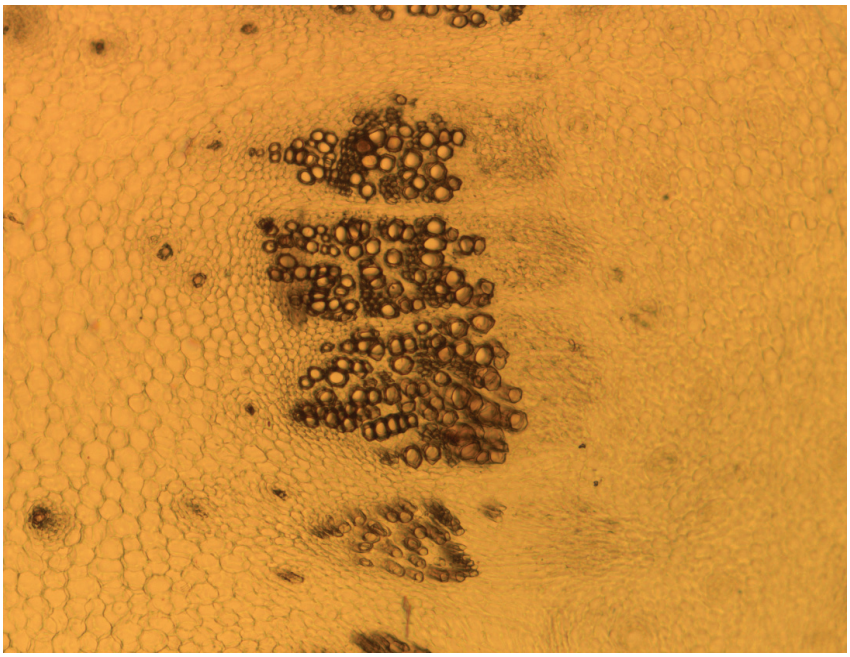

Figure 32. Transverse view of vascular bundles in *Coreopsis gigantea* (from 11.5 mm radius stem) (LM x 40).

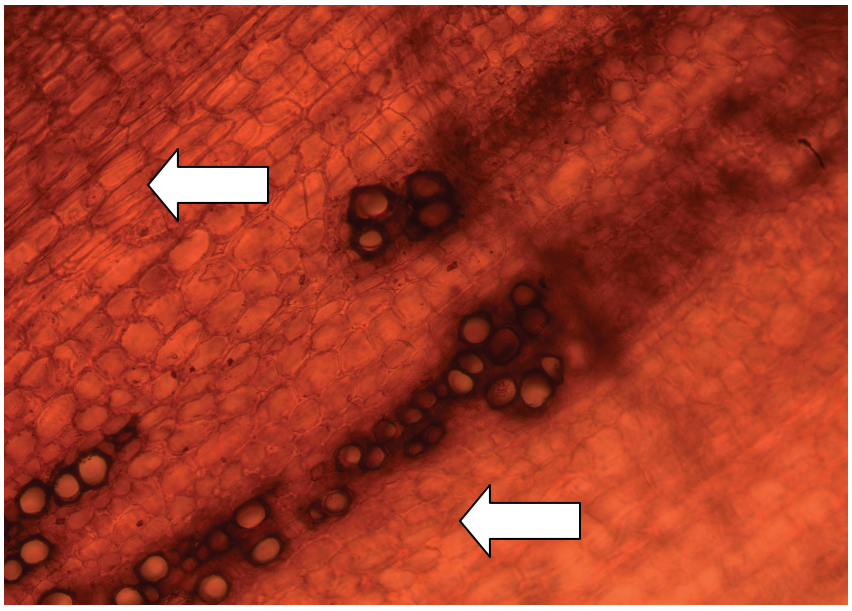

Figure 33. Transverse view of vascular bundles in *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x 100). The vascular cambial zone is the dark staining line at the xylem/phloem border. Arrows indicate parenchyma cells lying within interfasicular regions.

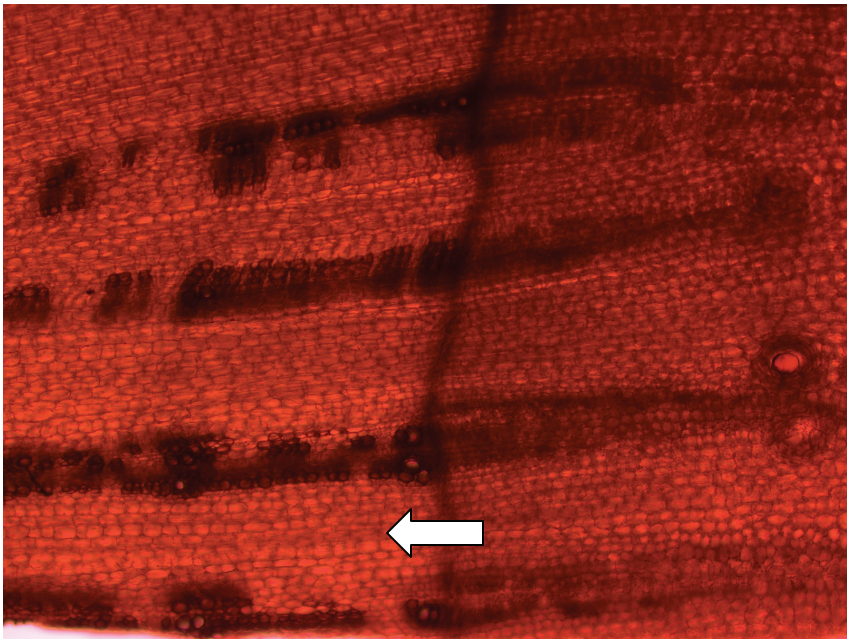

Figure 34. Transverse view of vascular bundles in *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x 40). The vascular cambial zone is the dark staining line at the xylem/phloem border. Arrow indicates parenchyma cells lying in an interfasicular region.

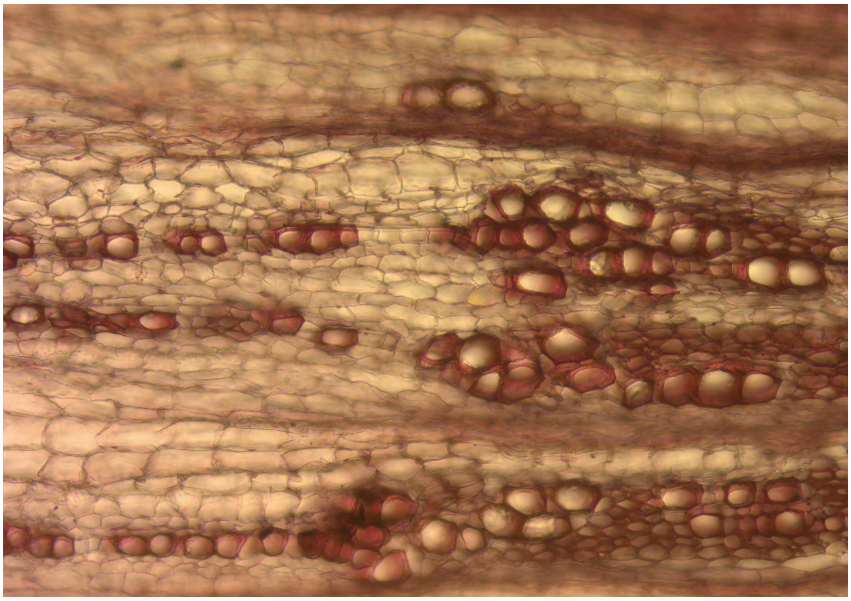

Figure 35. Transverse view of vascular bundles in *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x100).

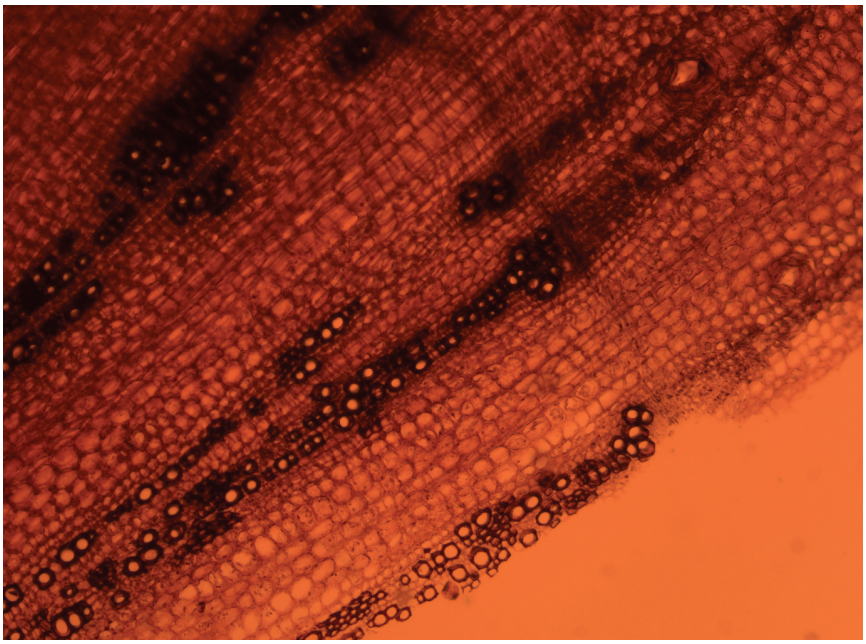

Figure 36. Transverse view of vascular bundles in *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x40). The vascular cambial zone is the dark staining line at the xylem/phloem border.

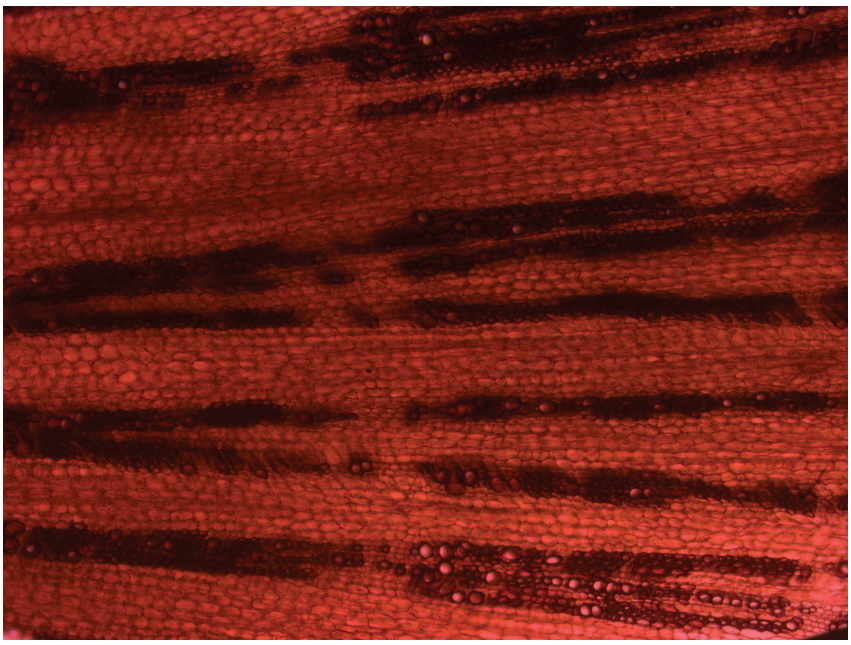

Figure 37. Transverse view of vascular bundles in *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x40).

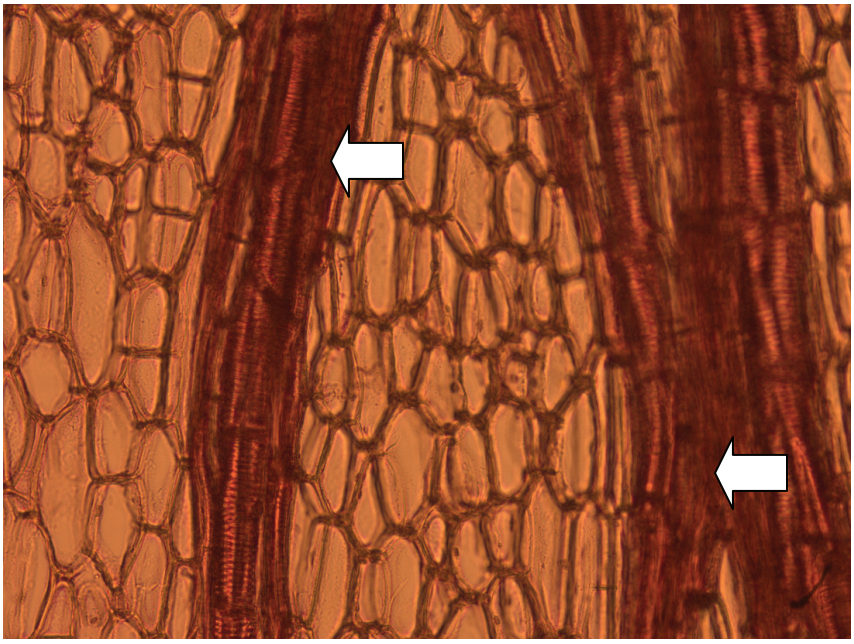

Figure 38. Tangential view of storied vessels (arrows) in *Coreopsis gigantea* (LM x100).

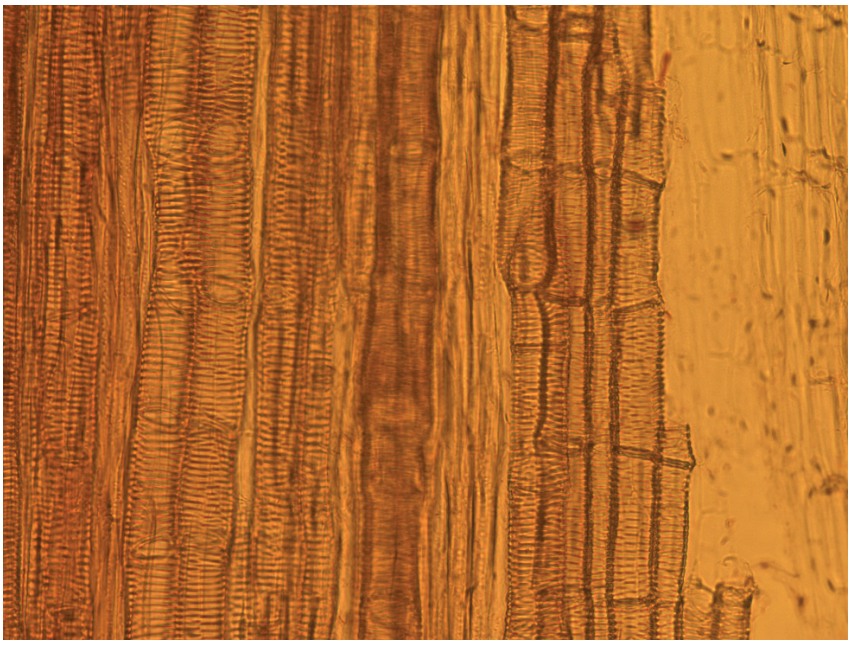

Figure 39. Radial view of storied vessels in *Coreopsis gigantea* (LM x100).

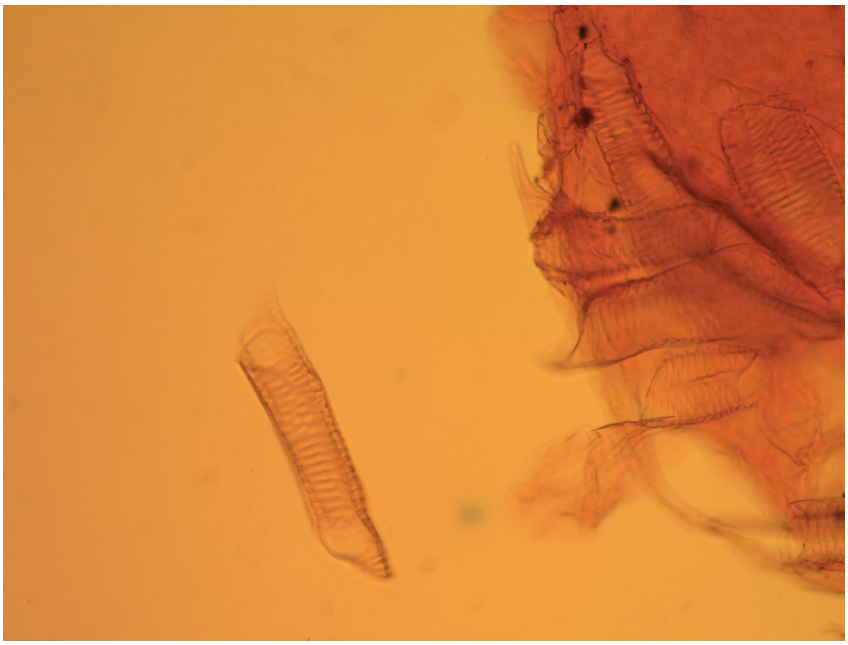

Figure 40. Vessel elements with caudate tips in *Coreopsis gigantea* (LM x200).

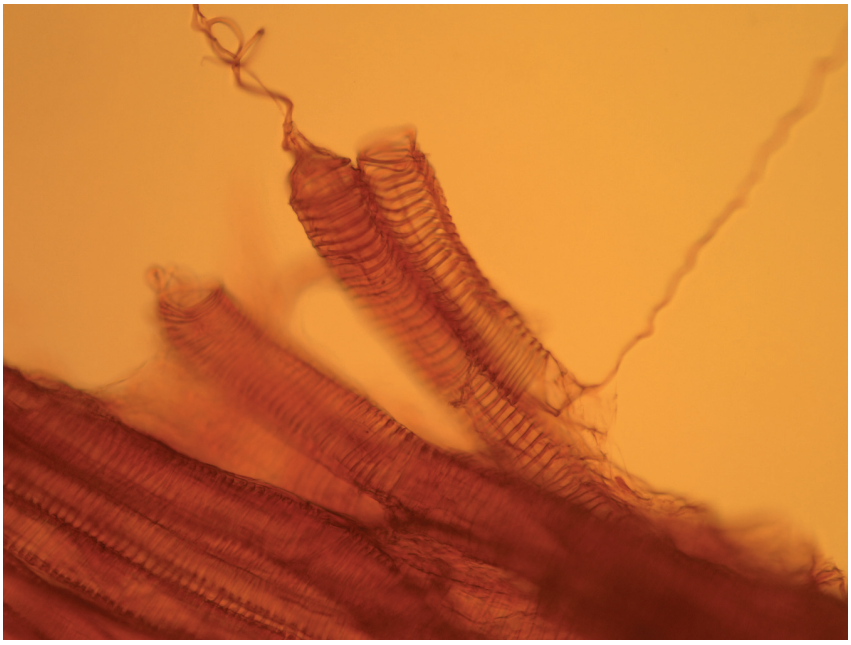

Figure 41. Vessel elements with a helical lateral wall deposition pattern in *Coreopsis gigantea* (LM x200). End walls show dissection damage resulting in unraveled helices.

Figure 42. Vessel element with slightly oblique end wall in *Coreopsis gigantea* (LM x200).

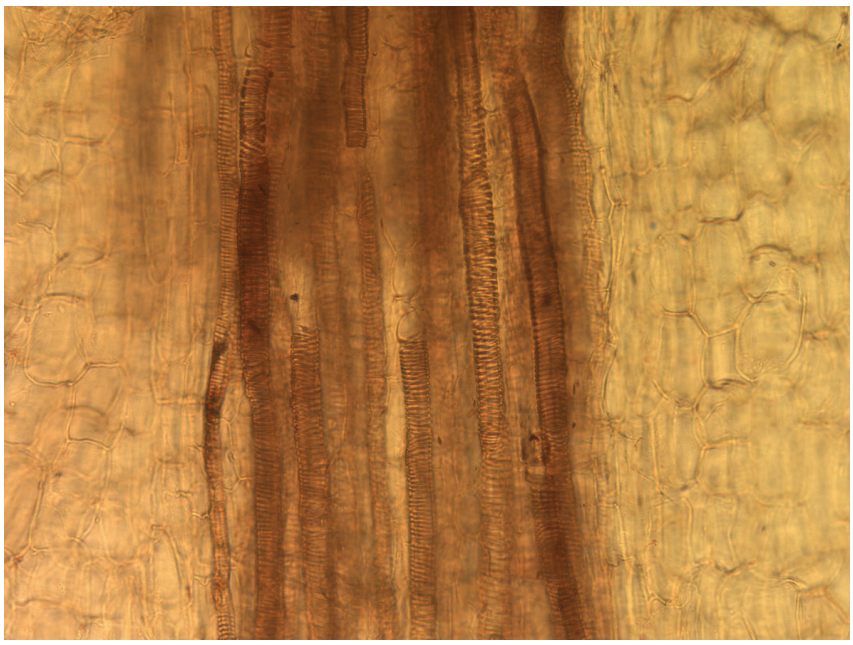

Figure 43. Radial view of parenchyma cells around earlier formed vessels in *Coreopsis gigantea* (LM x100).

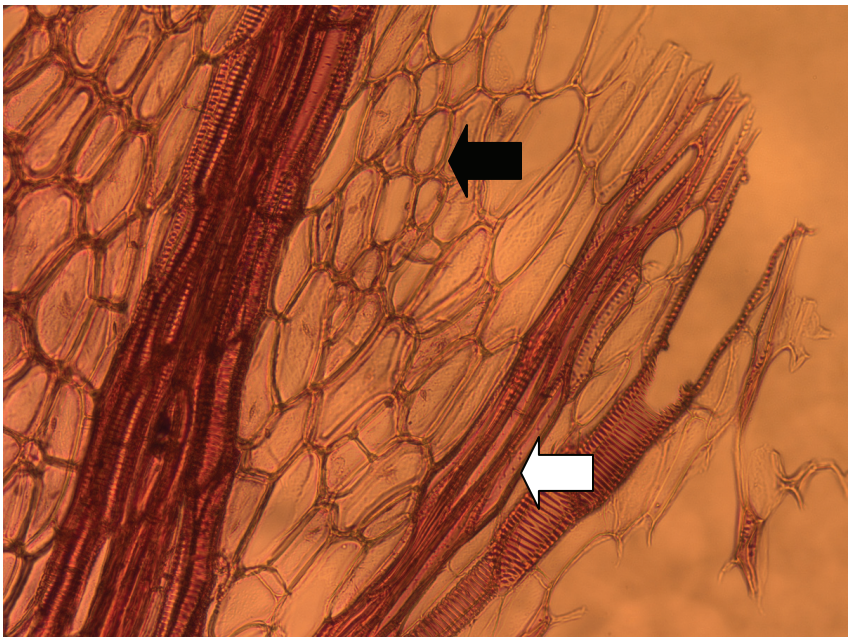

Figure 44. Tangential view of parenchyma cells (black arrow) in interfasicular regions of *Coreopsis gigantea*. A strand of libriform fibers (white arrow) lies to the right (LM x100).

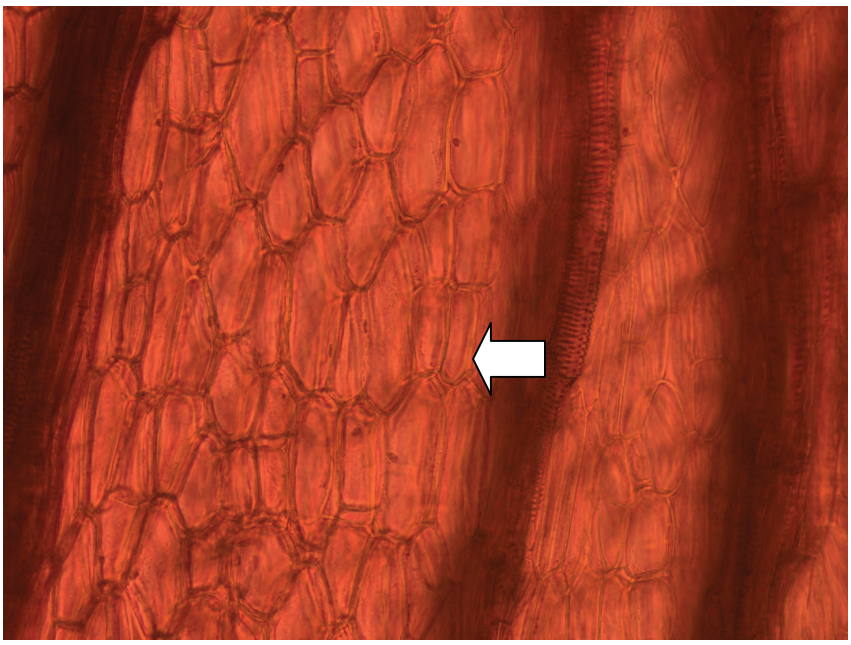

Figure 45.Tangential view of parenchyma cells (arrow) in the interfasicular regions of *Coreopsis gigantea* (LM x100).

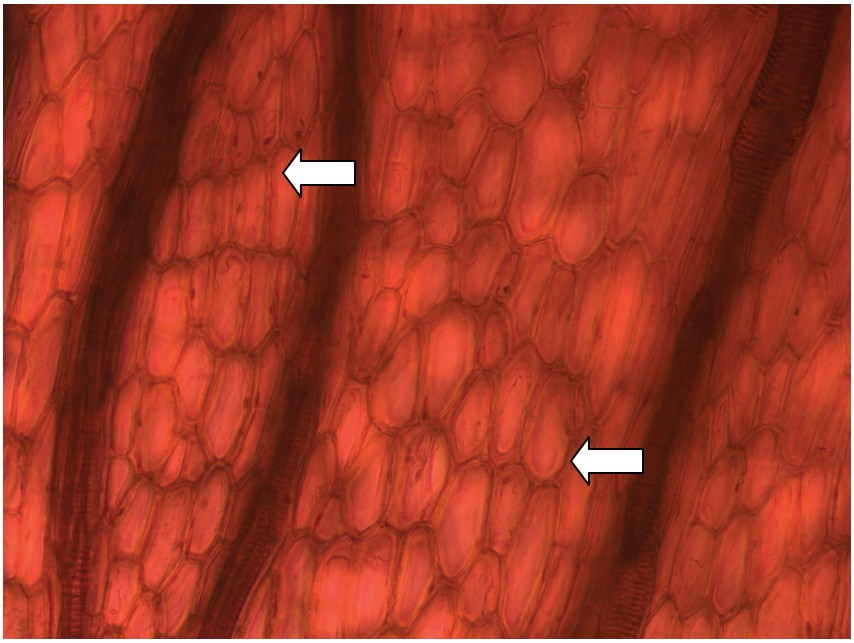

Figure 46. Tangential view of interfasicular regions (arrows) of *Coreopsis gigantea* (from 27.5 mm radius stem) (LM x100).

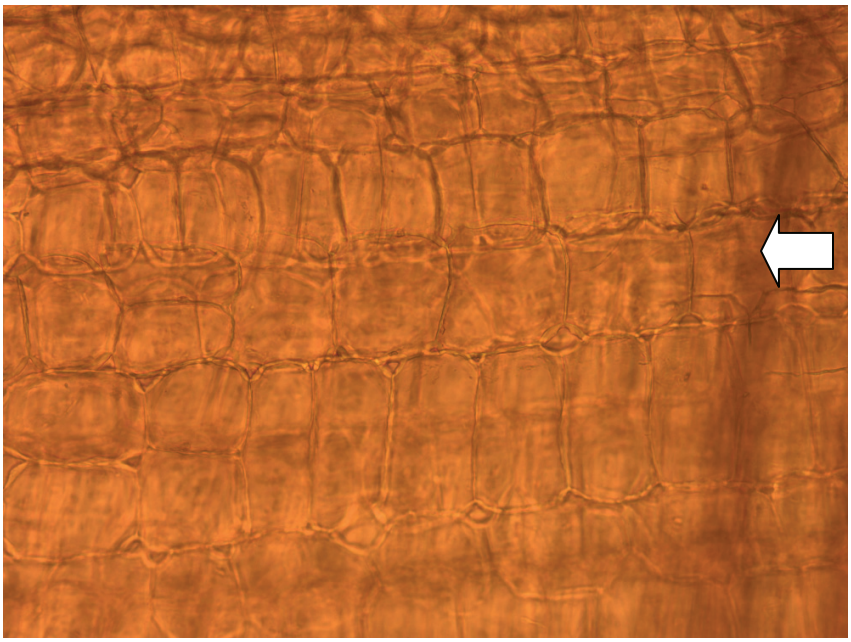

Figure 47. Radial view of the transition to upright parenchyma cells around the more recently formed vessels in *Coreopsis gigantea* (LM x100). Arrow points to area of new vessels.

Figure 48. Radial view of upright parenchyma cells (with a few procumbent cells) next to recently formed vessels in *Coreopsis gigantea* (LM x100).

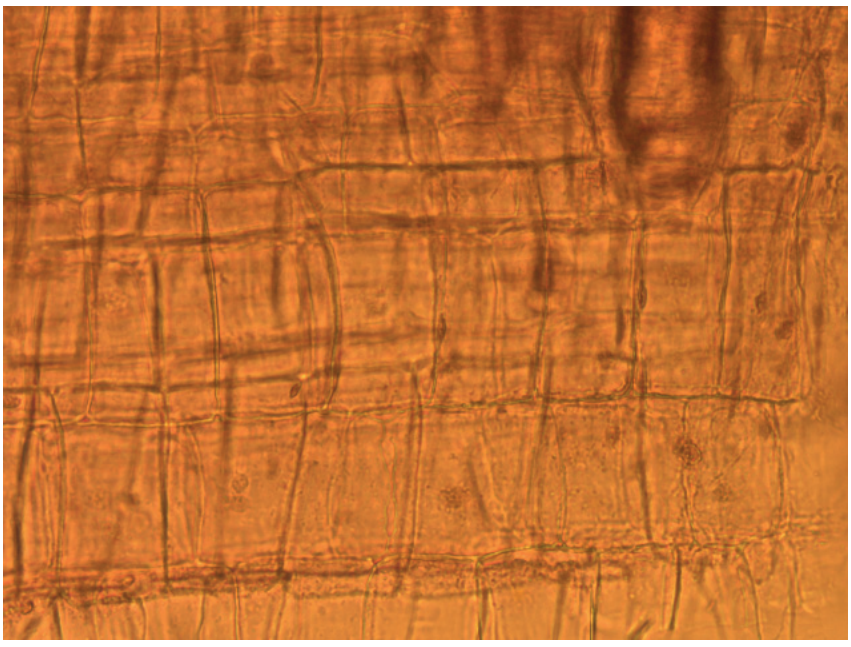

Figure 49. Radial view of upright parenchyma cells inside the vascular cambial zone in *Coreopsis gigantea* (LM x100).

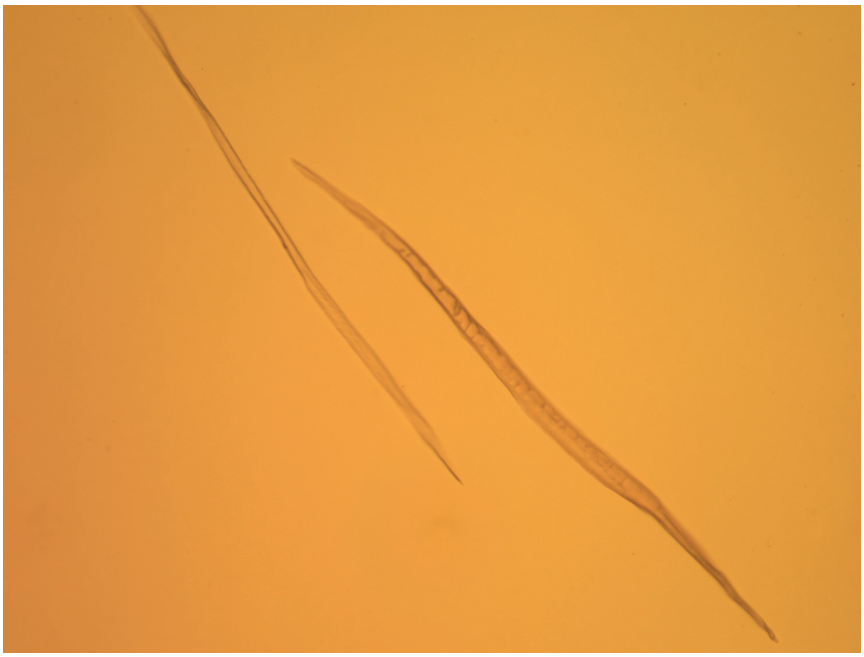

Figure 50. *Coreopsis gigantea* libriform fibers (LM x200).

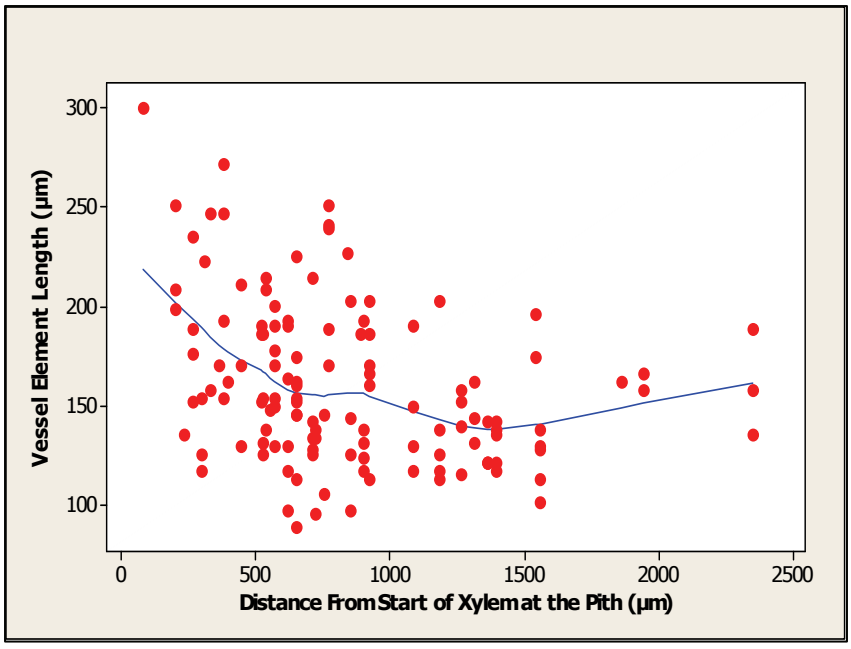

Figure 51. Change in vessel element length across the xylem of *Coreopsis gigantea* (small young stems) (Locally Weighted Scatterplot Smoother line fitted).

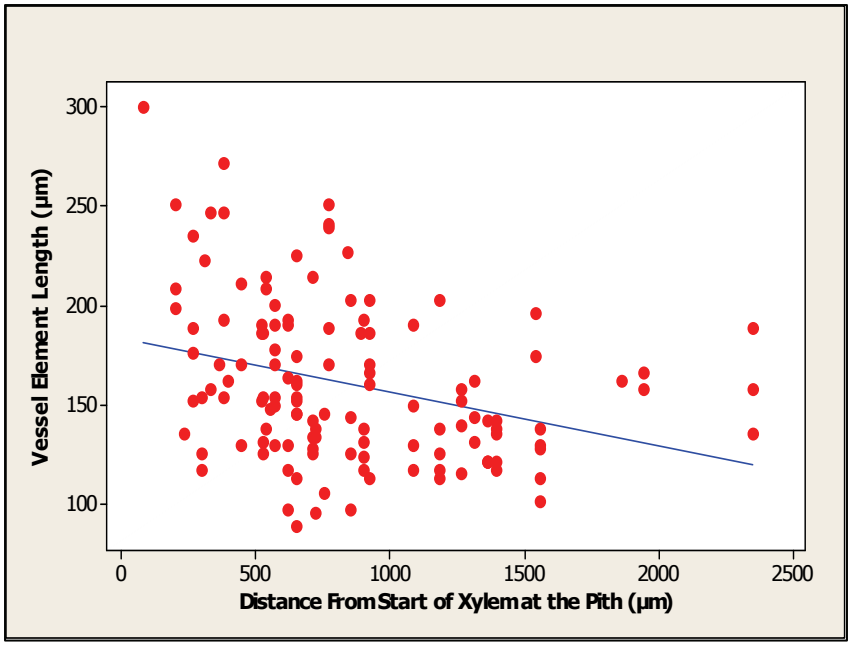

Figure 52. Change in vessel element length across the xylem of *Coreopsis gigantea* (small young stems) (linear regression line fitted).(p=0.0; r^2=10.1%)

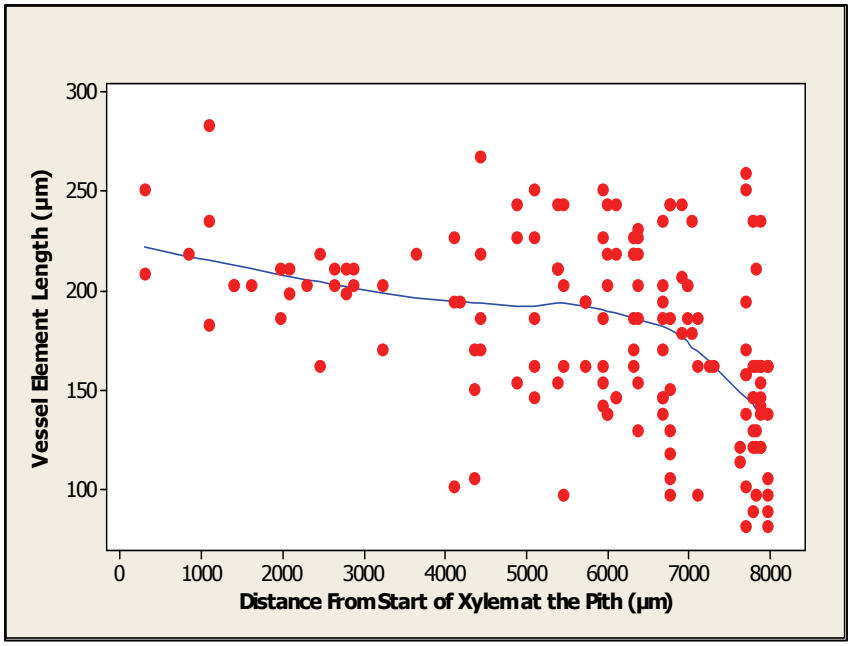

Figure 53. Change in vessel element length across the xylem of *Coreopsis gigantea* (29.0 mm radius stems) (Lowess line fitted).

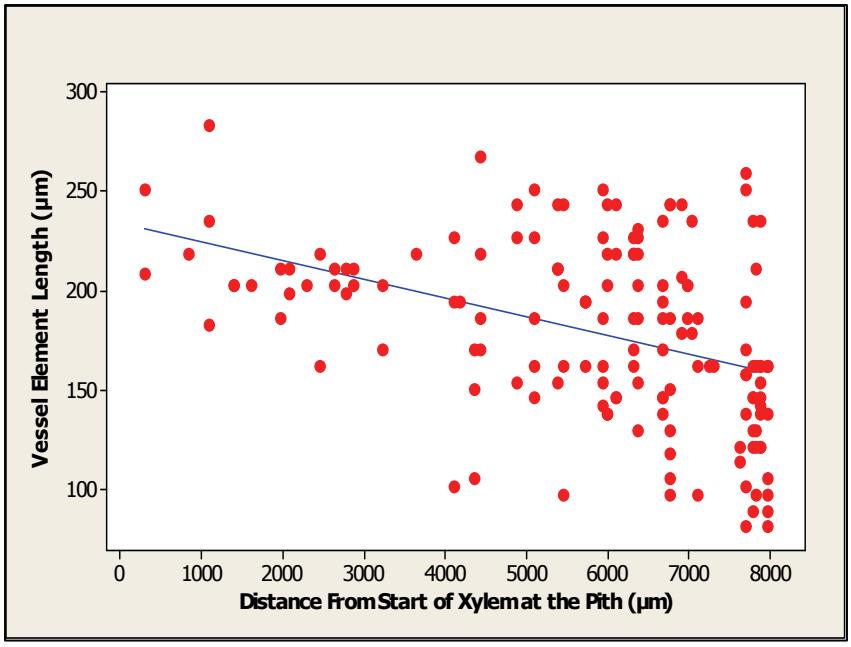

Figure 54. Change in vessel element length across the xylem of *Coreopsis gigantea* (29.0 mm radius stems) (linear regression line fitted). ($p = 0.0$; $r^2 = 17.5\%$)

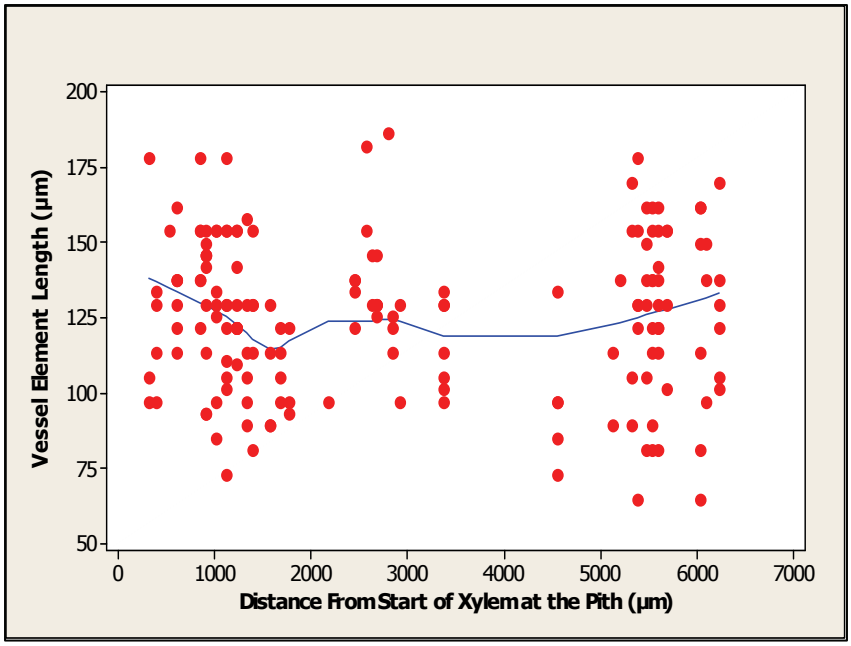

Figure 55. Change in vessel element length across the xylem of *Coreopsis gigantea* (second data set using 29.0 mm radius stems).

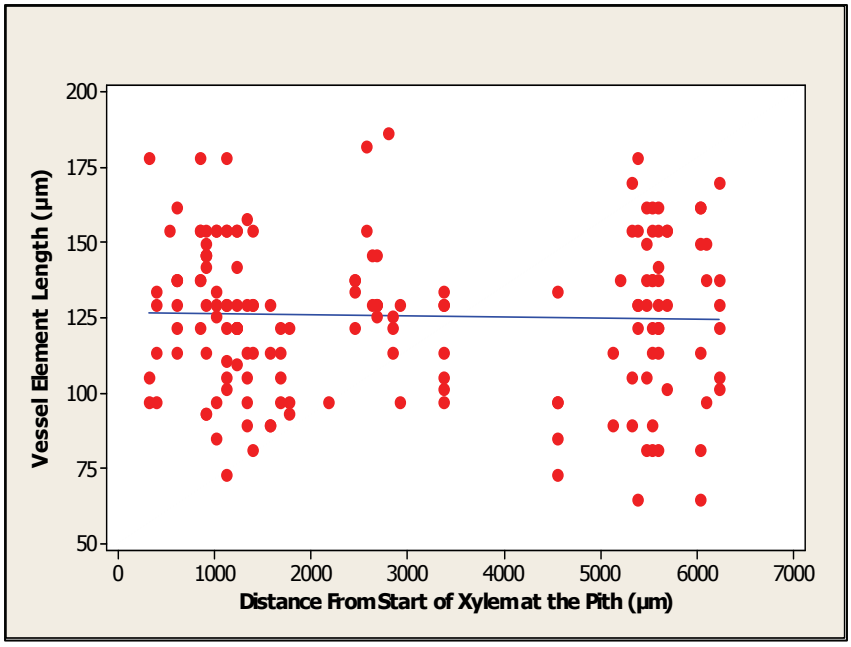

Figure 56. Change in vessel element length across the xylem of *Coreopsis gigantea* (second data set using 29.0 mm radius stems) (linear regression line fitted). ($p = 0.666$; $r^2 = 0.1\%$)

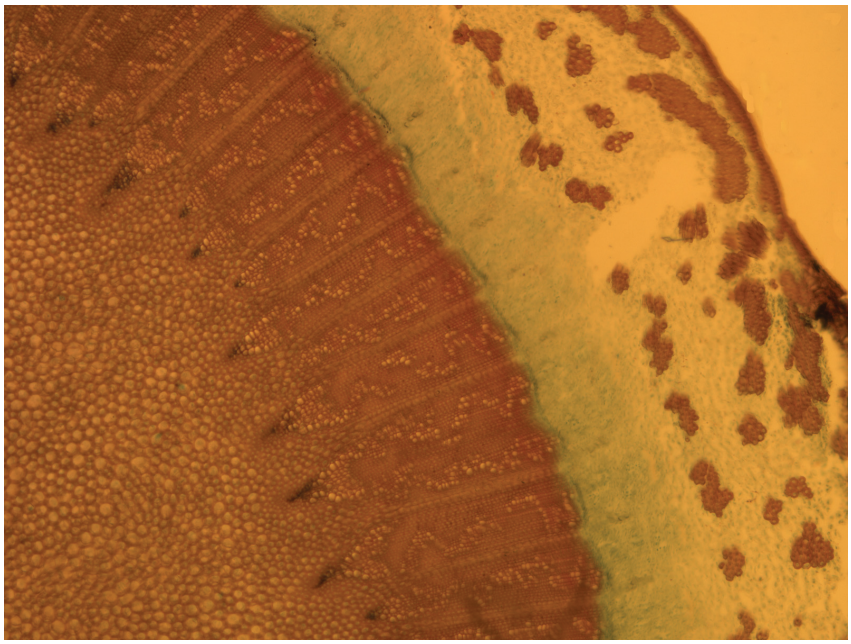

Figure 57. Transverse view of *Mahonia bealei* stem (LM x40).

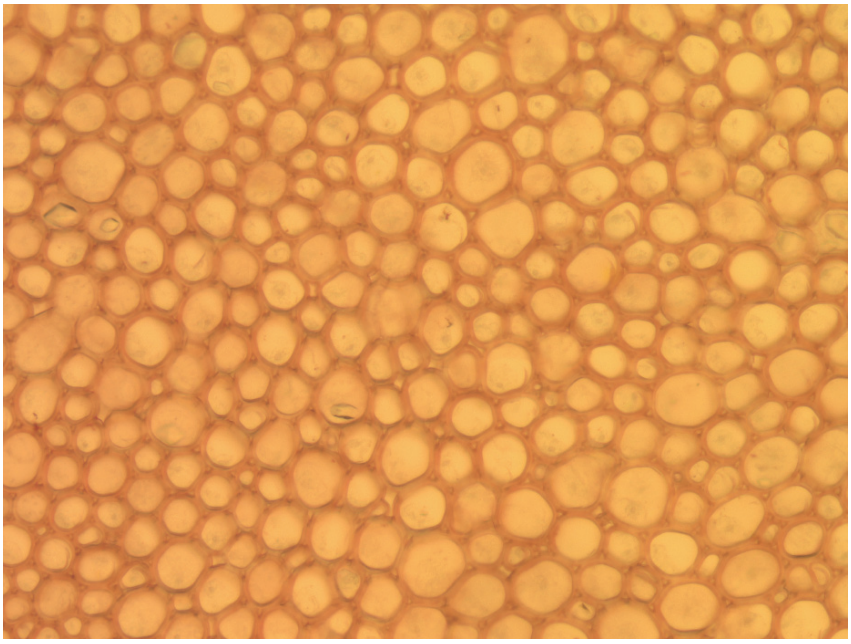

Figure 58. Transverse view of the pith cells in *Mahonia bealei* (LM x100).

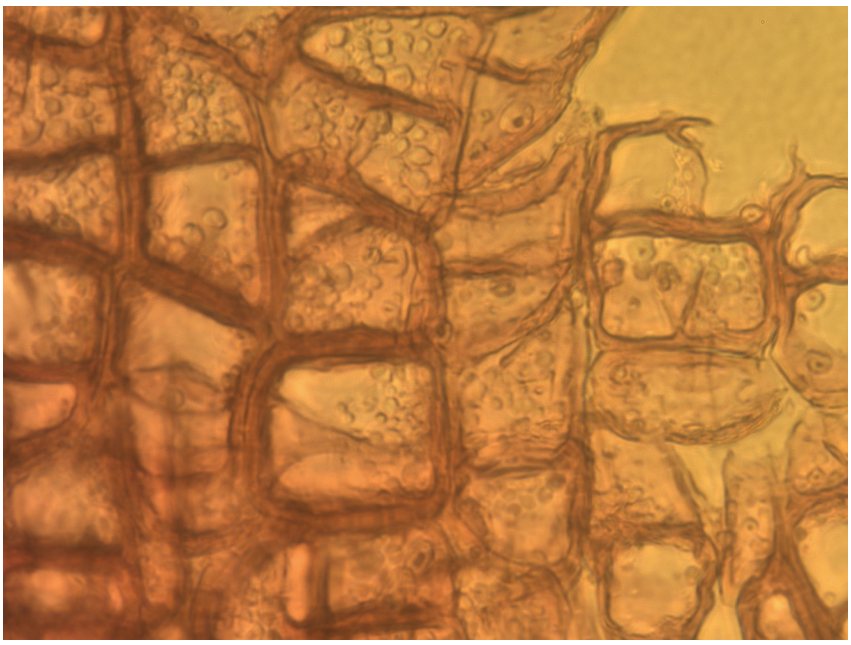

Figure 59. Tangential view of pith cells in *Mahonia bealei* (LM x400).

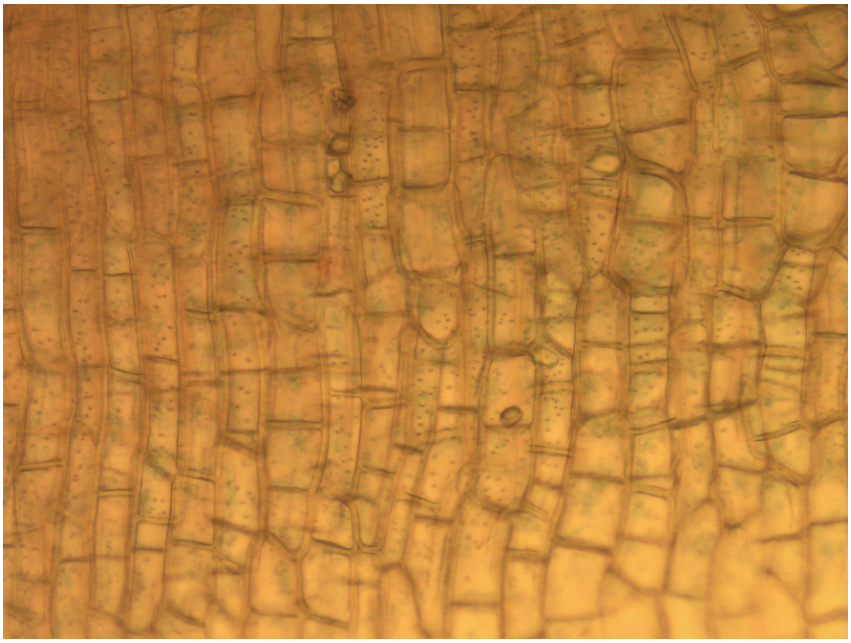

Figure 60. Radial view of pith cells in *Mahonia bealei* (LM x100).

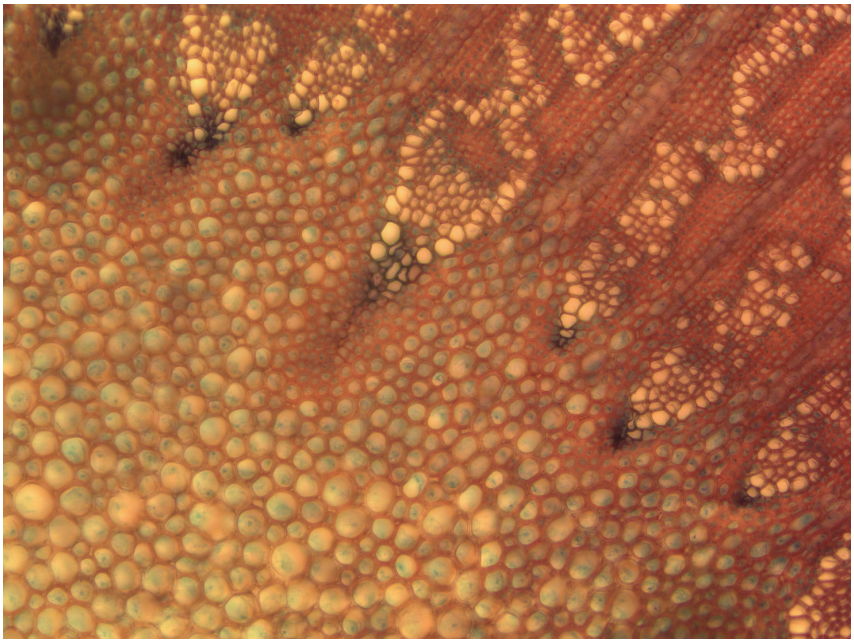

Figure 61. Transverse view of the earliest formed xylem in *Mahonia bealei*, next to the pith (LM x100).

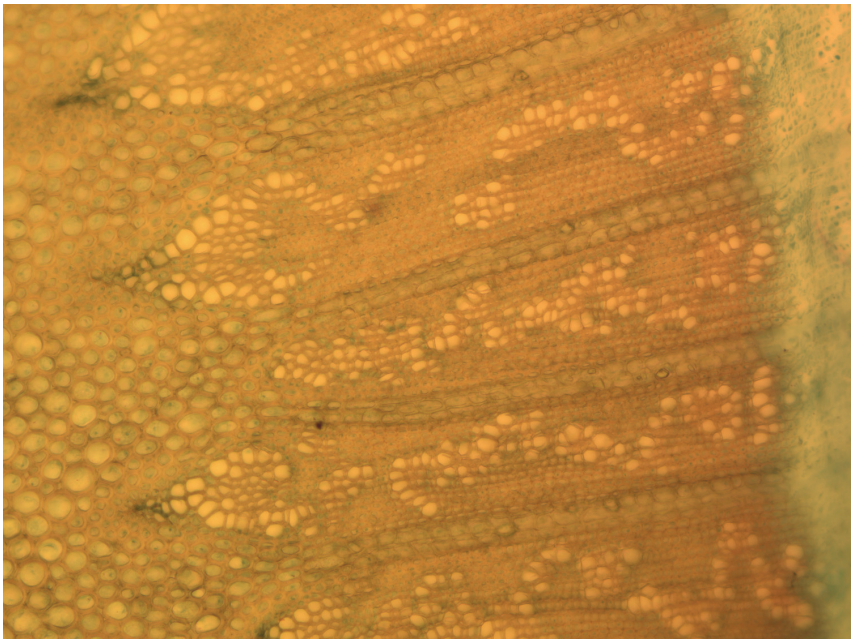

Figure 62. Transverse view of the secondary xylem in *Mahonia bealei* (LM x100).

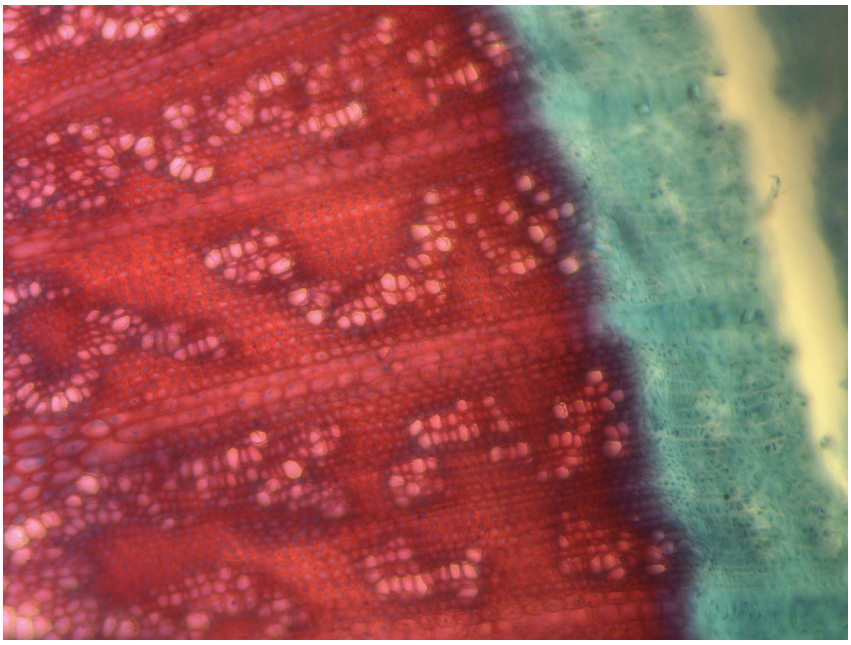

Figure 63. Transverse view of the secondary xylem in *Mahonia bealei* (LM x100).

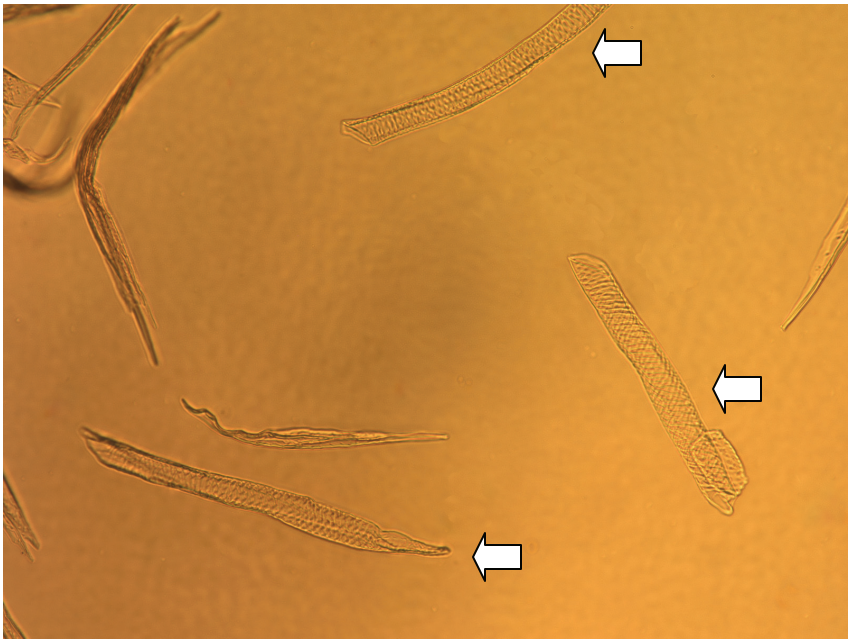

Figure 64. Three vessel elements (arrows) from *Mahonia bealei* (LM x200).

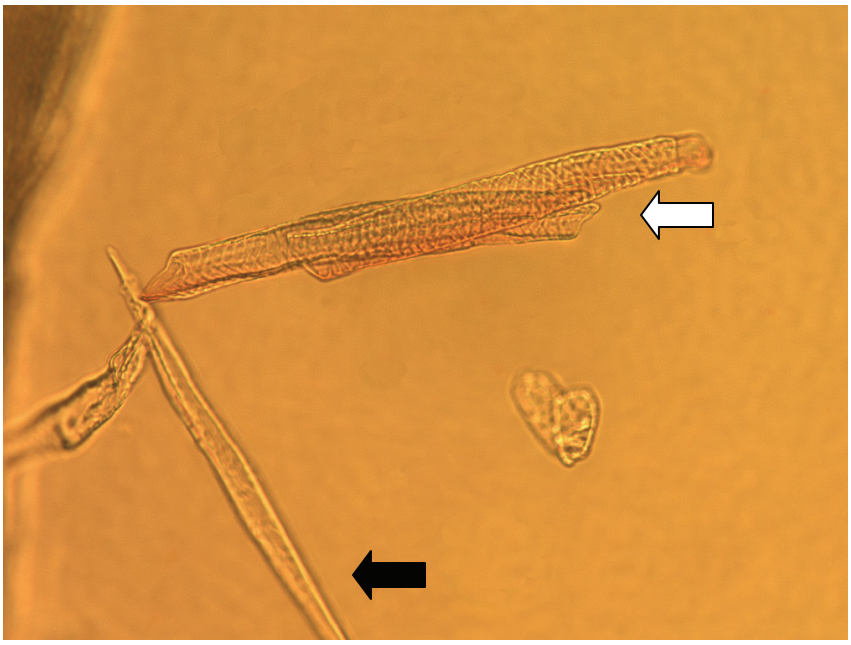

Figure 65. Two vessel elements (white arrow) beside a libriform fiber (black arrow) from *Mahonia bealei* (LM x200).

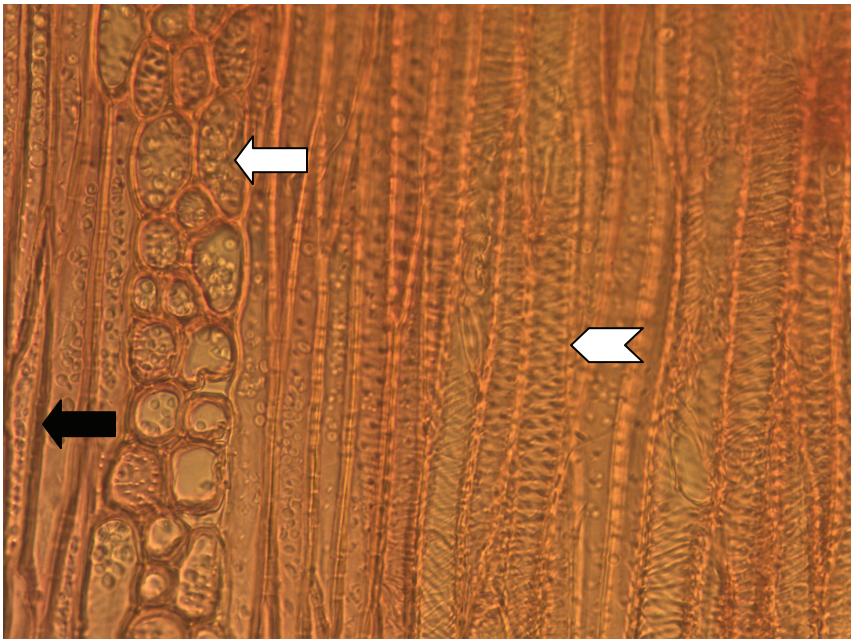

Figure 66. Tangential view of ray (white arrow), along with fibers (black arrow), and vessels (arrowhead) in *Mahonia bealei* (LM x40).

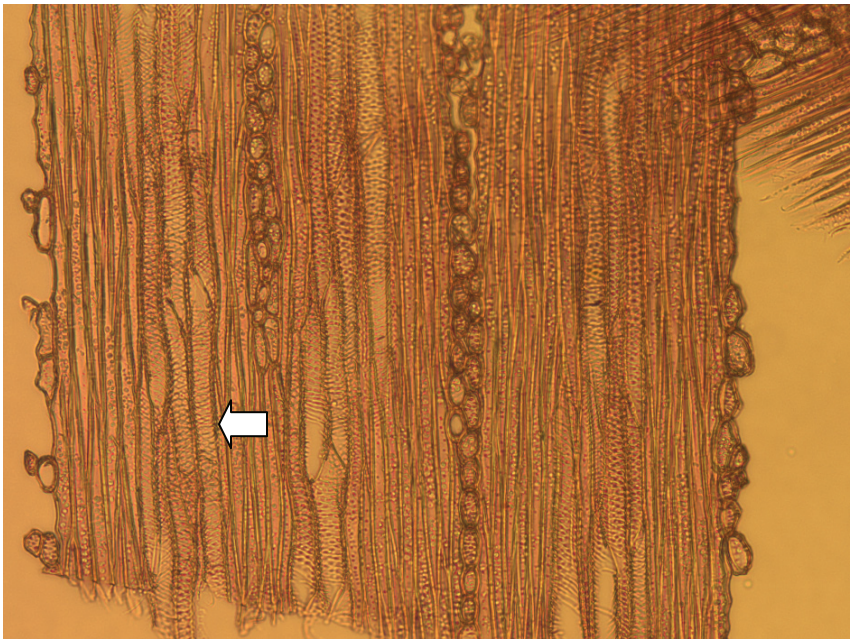

Figure 67. Tangential view of xylem from *Mahonia bealei* showing vessels with variable storying (arrow) (LM x100).

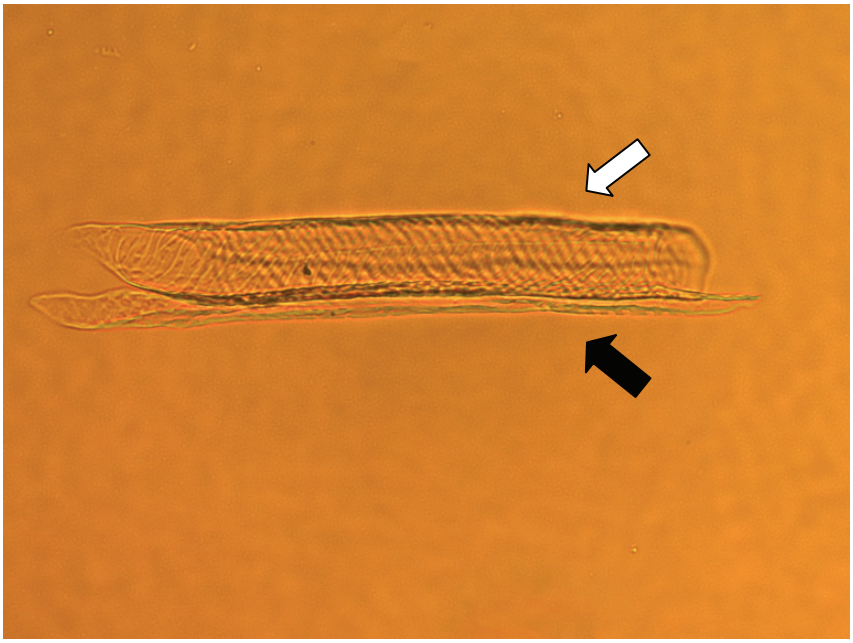

Figure 68. Vessel element (white arrow) from *Mahonia bealei*, with a tracheid (black arrow) beneath it (LM x200).

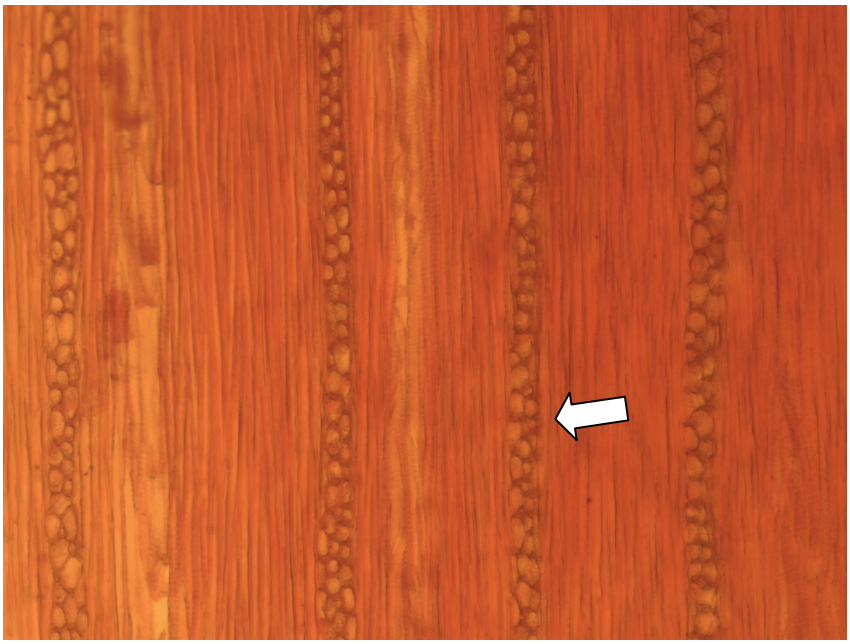

Figure 69. Tangential view of rays (arrow) in the secondary xylem of *Mahonia bealei* (LM x100).

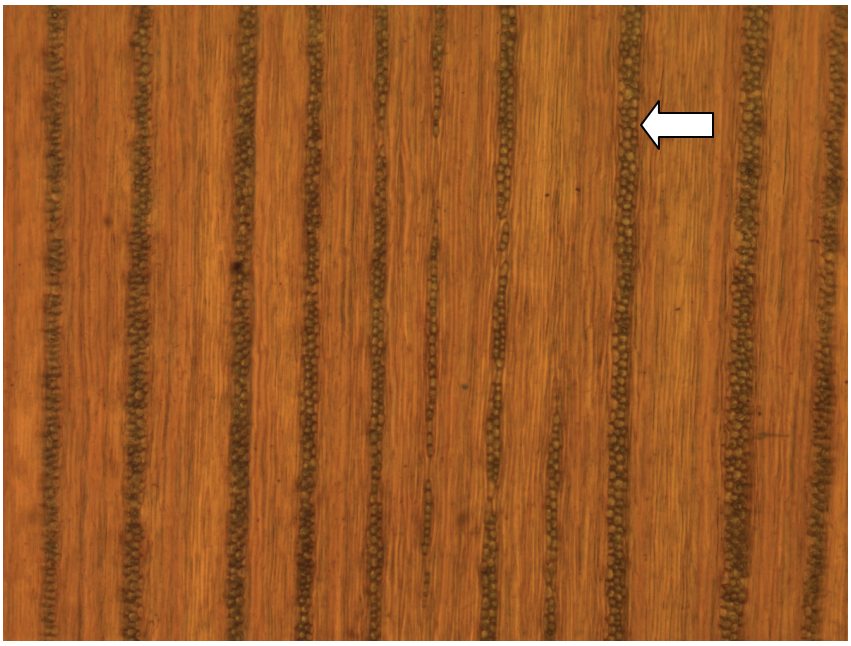

Figure 70. Tangential view of rays (arrow) in the secondary xylem of *Mahonia bealei* (LM x40).

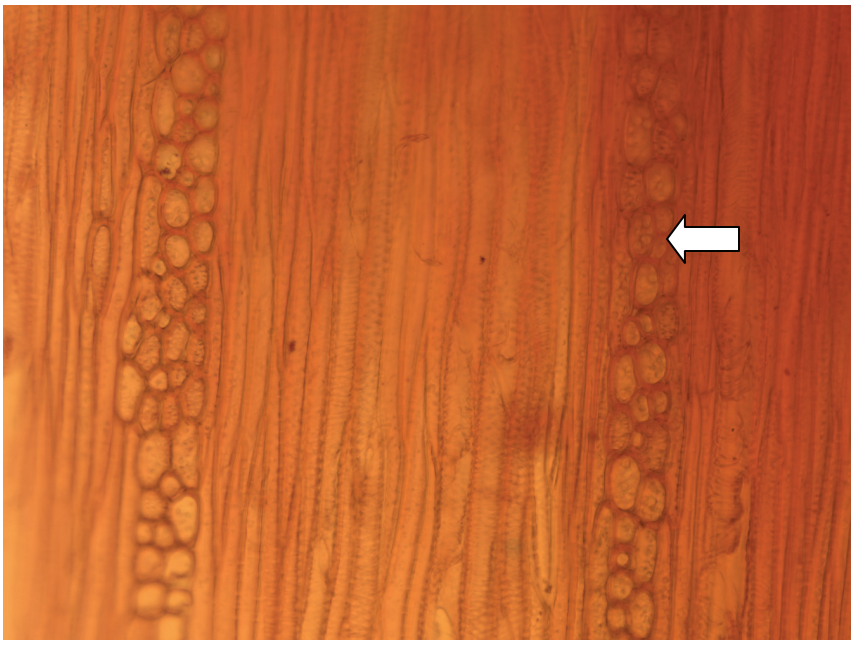

Figure 71. Tangential view of rays (arrow) in the secondary xylem of *Mahonia bealei* (LM x200).

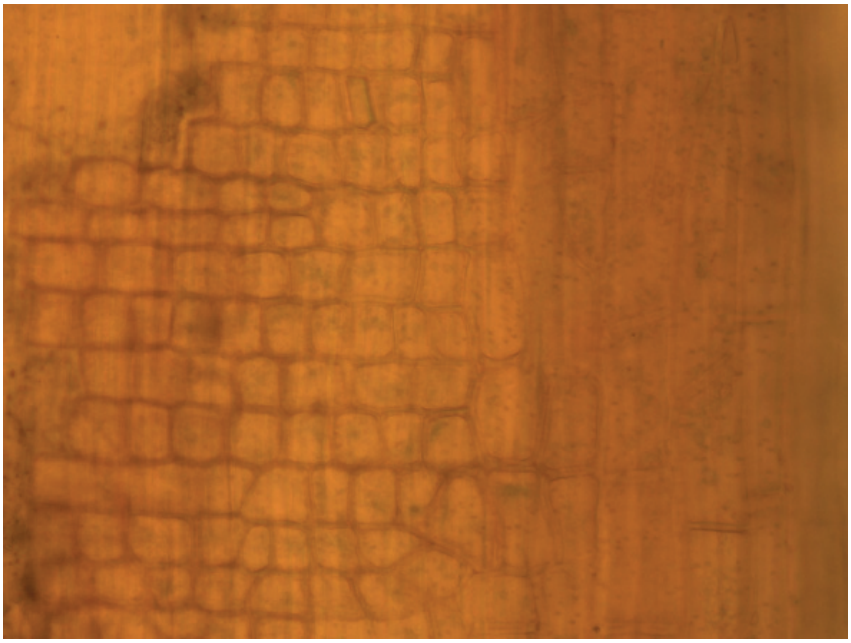

Figure 72. Radial view of a ray in the secondary xylem of *Mahonia bealei* (LM x100). Ray has a mix of upright (square cells are considered equivalent to upright cells) and procumbent cells.

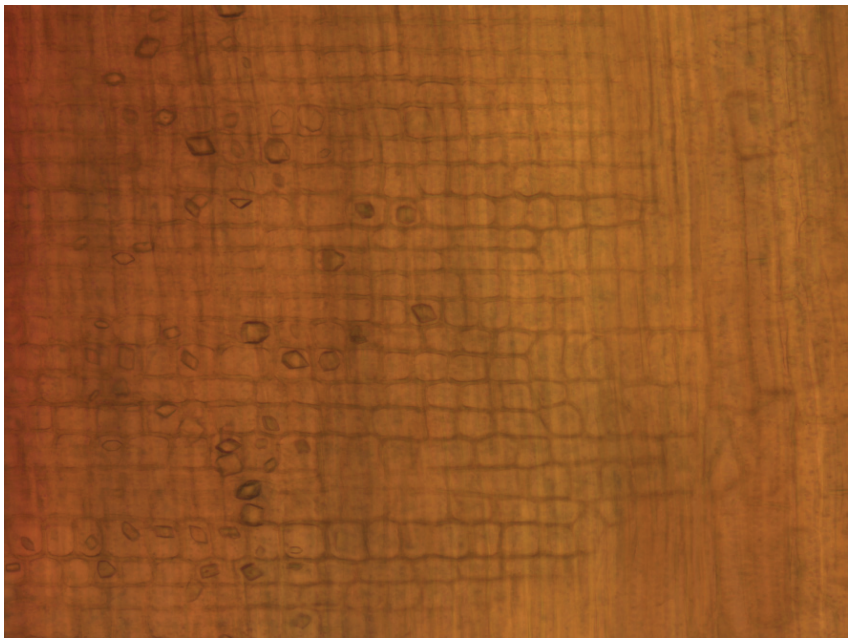

Figure 73. Radial view of a ray in the secondary xylem of *Mahonia bealei*. Rhomboid shaped crystals can be seen (LM x100).

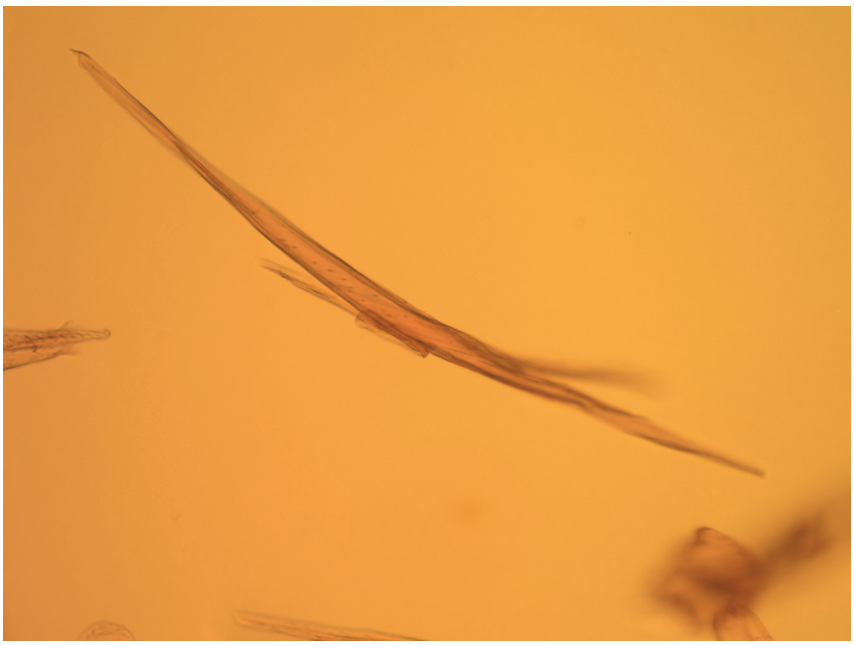

Figure 74. *Mahonia bealei* libriform fiber (LM x200).

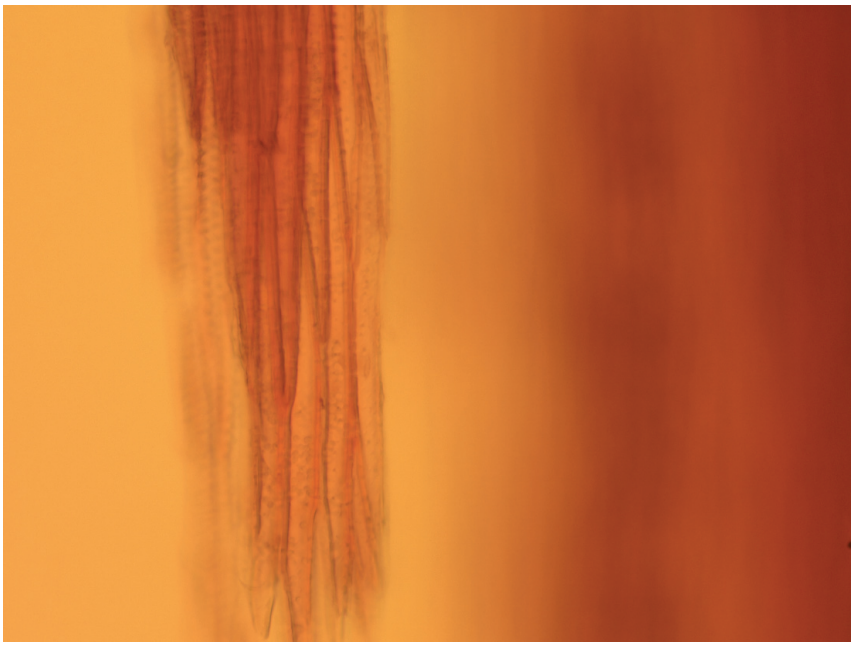

Figure 75. Example of limited storying in libriform fibers in *Mahonia bealei*. Fibers are basically nonstoried (LM x200).

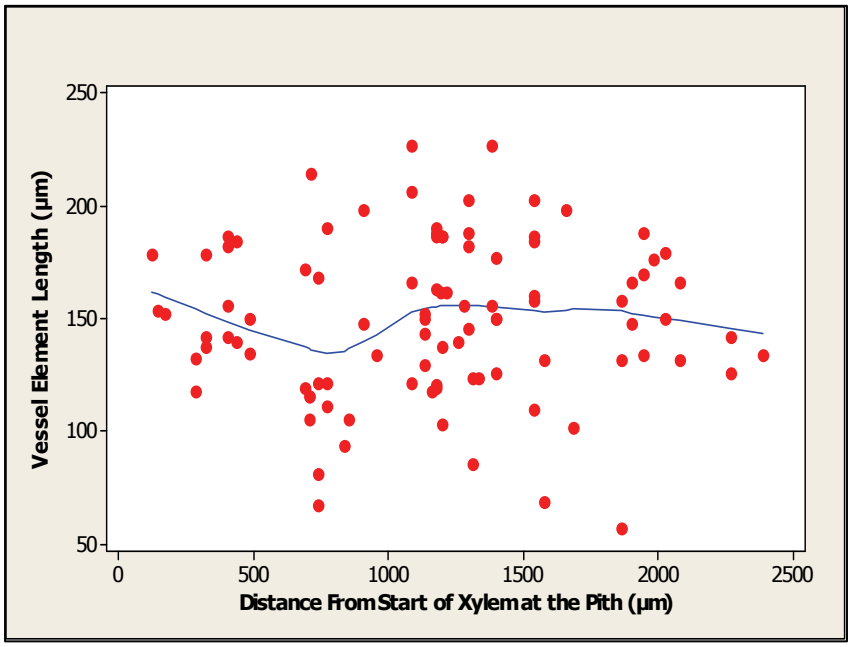

Figure 76. Change in vessel element length across the xylem of *Mahonia bealei* (Locally Weighted Scatterplot Smoother line fitted).

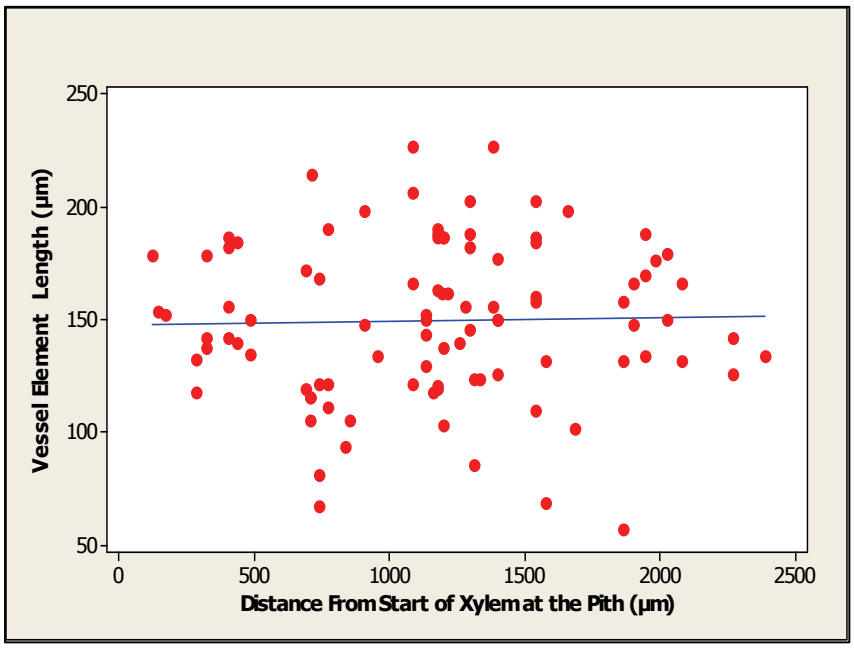

Figure 77. Change in vessel element length across the xylem of *Mahonia bealei* (linear regression line fitted). (p=0.832; r^2=0.0%)

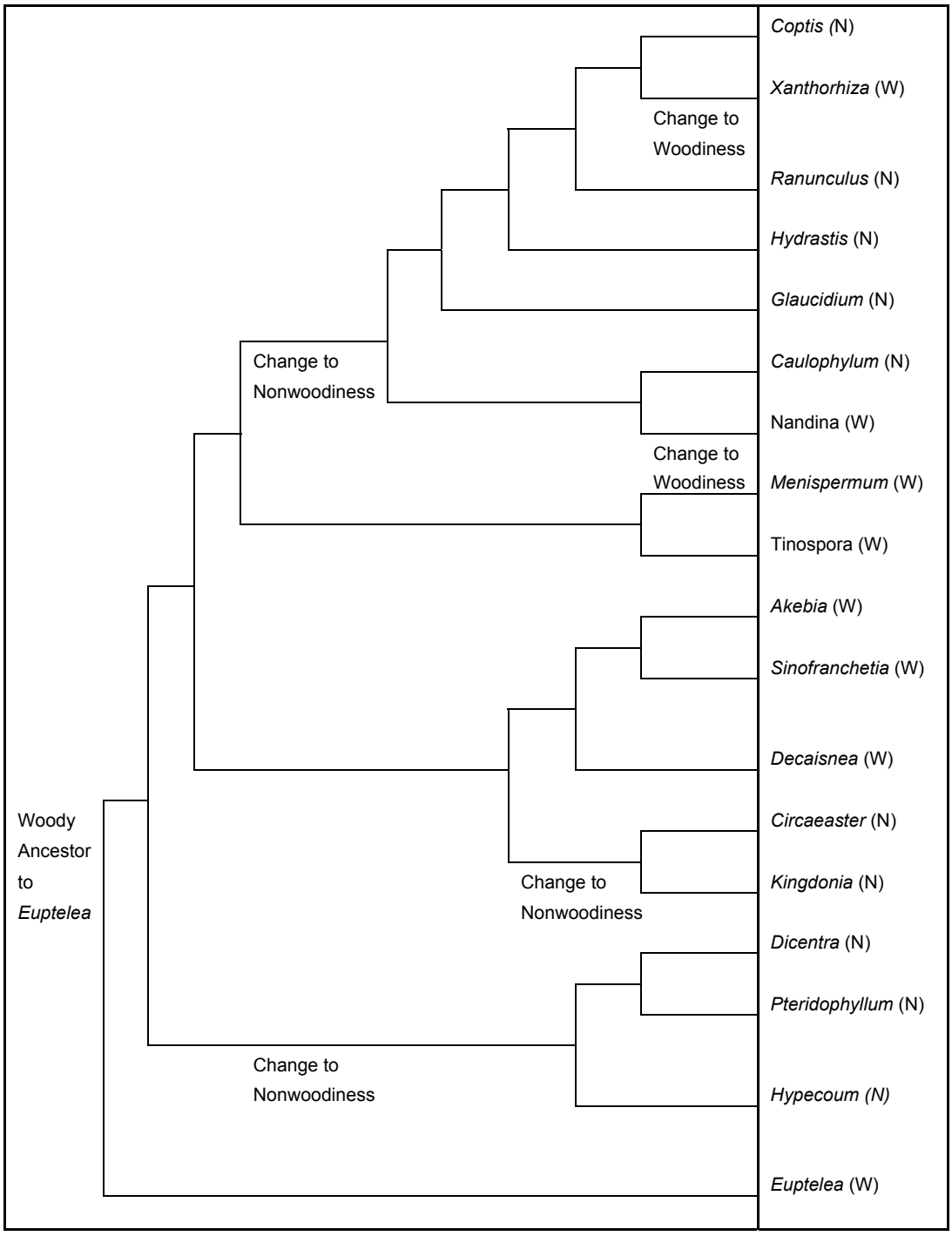

Figure 78. Phylogenetic relationships among genera of Ranunculales. Tree is based on an analysis of a four gene data set (18S rDNA, 26S rDNA, rbcL, and atpB) (Kim et al. 2004). W=woody; N=nonwoody.

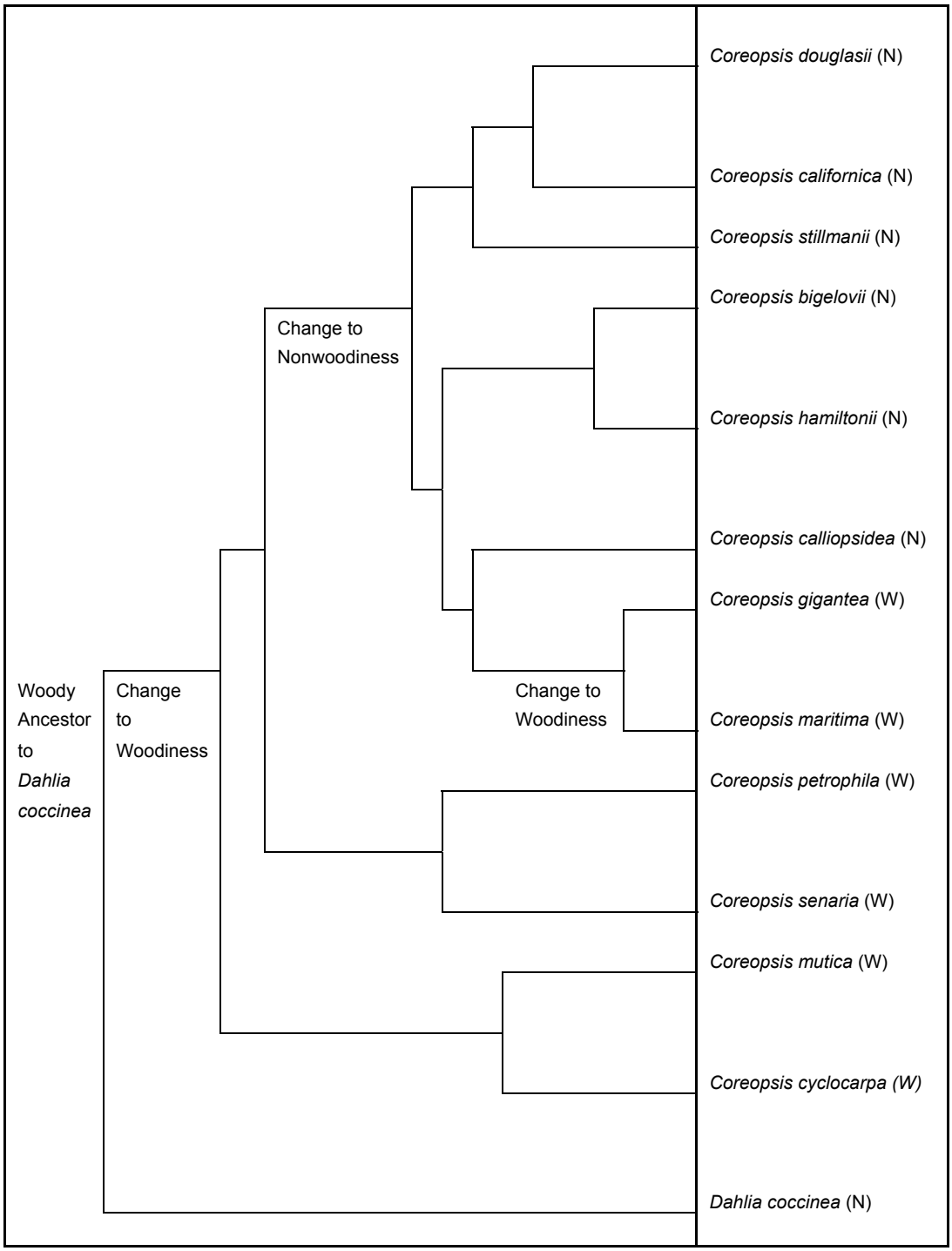

Figure 79. Phylogenetic relationships among *Coreopsis* species. Tree is based on an analysis of ITS-1, ITS-2, and rpl 16 sequences (Archibald et al. 2005). W=woody; N=nonwoody.

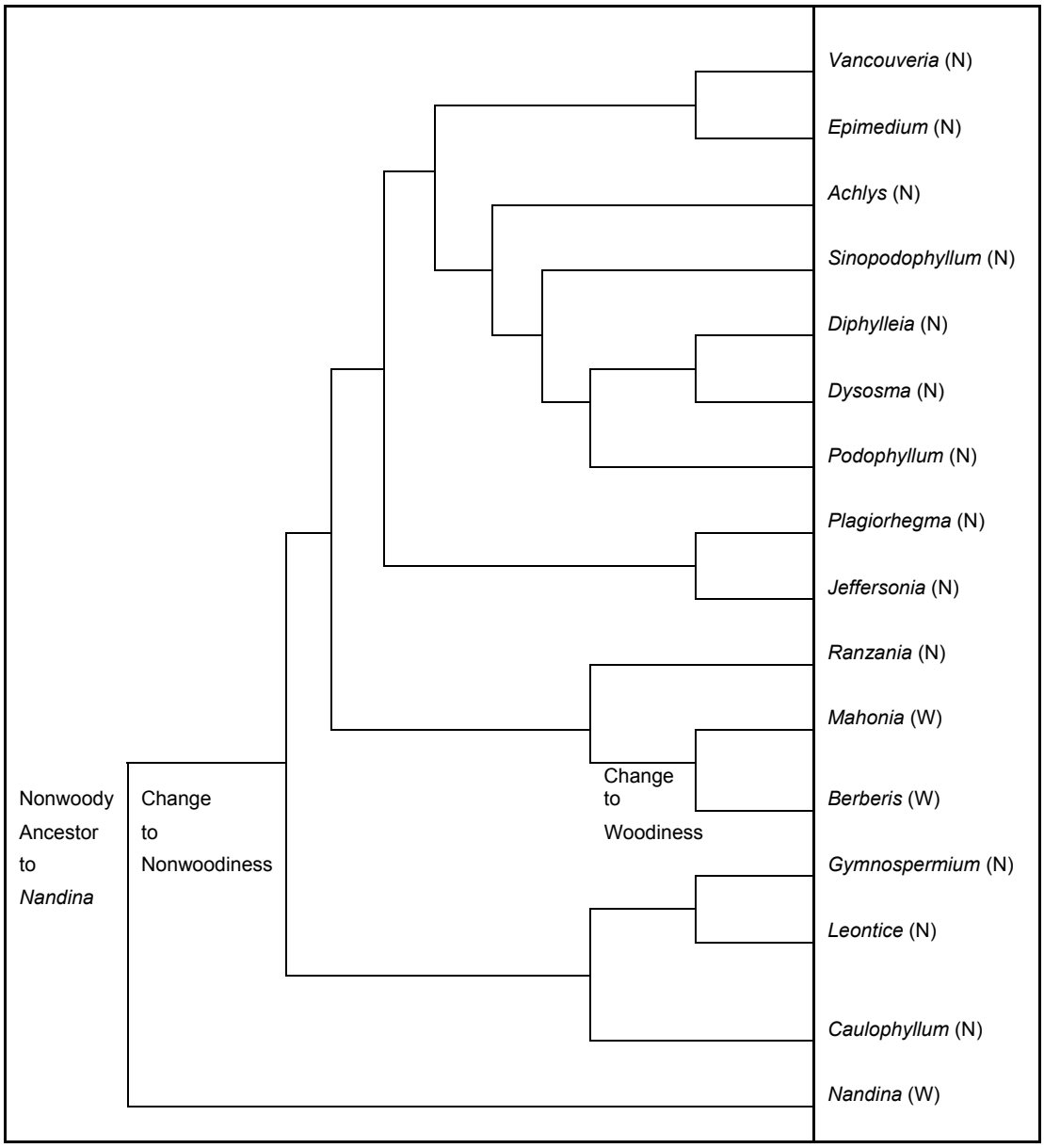

Figure 80. Phylogenetic relationships among genera of Berberidaceae. Tree is based on an analysis of chloroplast DNA restriction site data (Kim and Jansen 1998). W=woody; N=nonwoody.